

SCIENCE ET TECHNIQUE DU FROID

**Aptitude à la conservation
des poissons et produits
de la mer réfrigérés et congelés**

**Storage lives
of chilled and frozen fish
and fish products**

INSTITUT INTERNATIONAL DU FROID
INTERNATIONAL INSTITUTE OF REFRIGERATION

Commissions C2 & D3

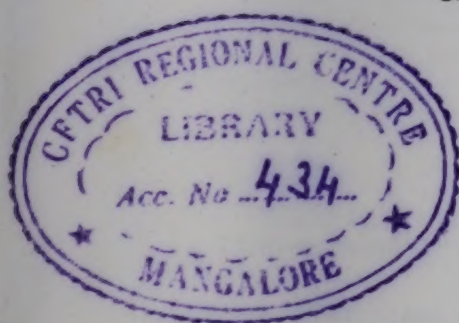
Aberdeen (United Kingdom)

1985-4



**Aptitude à la conservation
des poissons et produits
de la mer
réfrigérés et congelés**

**Storage lives
of chilled and frozen fish
and fish products**



Compte rendu des réunions de:
Proceedings of meetings of:

Commissions C2 & D3

(Oct. 1-3, 1985)

Édité par / *Issued by*

INSTITUT INTERNATIONAL DU FROID
INTERNATIONAL INSTITUTE OF REFRIGERATION

177, Boulevard Malesherbes, F-75017 Paris (France)



Attitude à la conservation
des poissons et produits
de la mer
réfrigérés et congelés

Storage lives
of chilled and frozen fish
and fish products



La reproduction totale ou partielle de tout ce qui paraît dans ce livre est autorisée sous réserve de la citation précise de la source originale.

Les opinions émises dans cette publication n'engagent que leurs auteurs.

For the full or partial reproduction of anything published in this book proper acknowledgement should be made to the original source.

Any opinions expressed herein are entirely those of the authors.

TABLES DES MATIERES - CONTENTS

Avant-propos - Foreword	8-9
Welcoming speech, by Prof. J. Connell	12
Allocution par le Dr. A. Gac, Directeur de l'Institut International du Froid	14-15
Address by Dr. A. Gac, Director of the International Institute of Refrigeration	
Liste des participants - List of participants	17

EXPOSE INTRODUCTIF - INTRODUCTORY SPEECH

M. JUL (Denmark). Chilling and freezing fishery products ; changes in views and usages	23
Réfrigération et congélation des produits de la pêche ; évolutions dans la conception et la pratique	

COMMUNICATIONS

SECTION 1

Qu'entend-on par aptitude à l'entreposage ?	What is shelf life ?
R.J. LEARSON, J.J. LICCIARDELLO (U.S.A.). Literature reporting of shelf life data. What does it all mean ?	31
La littérature sur la conservation en magasin. Quelle en est la valeur ?	
L. HERBORG (Denmark). Shelf-life - Quality : a question of relativity	39
Aptitude à la conservation - Qualité : une question de relativité	
P. HOWGATE (United Kingdom). Approaches to the definition and measurement of the storage life of chilled and frozen fish : a brief review	45
A propos de la définition et de la mesure de la durée de conservation du poisson réfrigéré et du poisson congelé : brève revue	
M. SANDERSON-WALKER (United Kingdom). Time temperature monitoring and quality inspection for a quick frozen food manufacturer. Recent changes in design and practical considerations for fish products	55
Surveillance automatique de la durée et de la température et inspection de la qualité pour un fabricant d'aliments surgelés. Modifications récentes de la conception et considérations pratiques pour les produits à base de poisson	

SECTION 2

Evolution chimique au cours de l'entreposage de produits congelés	Chemical changes in frozen storage
SHANN-TZONG JIANG, CHING-YU TSAO, MING-LANG HO (Taiwan). Effect of the temperature fluctuation on the meat quality of frozen mackerel (<i>Scomber tapeinocephalus</i>)	63
Influence des fluctuations de température sur la qualité de la chair de maquereau (<i>Scomber tapeinocephalus</i>) congelé.	
T. BØRRESEN, L.B. KNUDSEN, J. NIELSEN (Denmark). Denaturation of sarcoplasmic proteins during frozen storage of fish	71
Dénaturation des protéines sarcoplasmiques au cours de l'entreposage du poisson à l'état congelé	

L. STODOLNIK (Poland). Lipid changes in cod muscle tissue frozen in liquid nitrogen during frozen storage	79
<i>Modifications des lipides du tissu musculaire de cabillaud congelé dans l'azote liquide au cours de l'entreposage</i>	
A.J. BORDERIAS, P. MONTERO (Spain). Changes in fish muscle collagen during frozen storage	85
<i>Modifications du collagène du tissu musculaire de poisson congelé pendant la conservation</i>	
H. REHBEIN (Fed. Rep. of Germany). Does formaldehyde form cross-links between myofibrillar proteins during frozen storage of fish muscle ? ...	93
<i>La formaldéhyde forme-t-elle des liaisons croisées entre les protéines myofibrillaires au cours de l'entreposage de muscle de poisson congelé ?</i>	

SECTION 3

Entreposage des poissons et produits de la mer congelés	Storage of frozen fish and shellfish
J. JOSEPH, P.A. PERIGREEN, M.R. NAIR (India). Effect of raw material quality on the shelf-life of frozen squid (<i>Loligo Duvaucellii</i>) mantles..	103
<i>Influence de la qualité de la matière première sur la conservation de manteaux de calmar (<i>Loligo duvaucellii</i>) congelé.</i>	
D.S. REID, N.F. DOONG, A. FOIN, M. SNIDER (U.S.A.). Studies on the frozen storage of fish	111
<i>Etudes de l'entreposage du poisson à l'état congelé.</i>	
D.J.B. DA PONTE, J.P. ROOZEN, W. PILNIK (Netherlands). Effect of different hydrocolloids on the stability of frozen stored minced fillets of whiting and cod	119
<i>Influence de divers hydrocolloïdes sur la stabilité de filets hachés de merlan et de cabillaud conservés à l'état congelé</i>	
K.W. YOUNG, A. CRAIG, P. HOWGATE, G.L. SMITH, K.J. WHITTLE (United Kingdom). The storage lives of various frozen minces and fillet of cod compared with five other gadoid species	125
<i>L'aptitude à l'entreposage de divers hachis et filets congelés de cabillaud comparée avec celle de cinq autres espèces de gadoides</i>	
T.T. KOZIMA (Japan). Frozen storage of fish and fish products in Japan and novel dehydrofreezing of fish	133
<i>Entreposage à l'état congelé du poisson et des produits de la pêche au Japon et nouvelle méthode de déshydratation-congélation du poisson</i>	

SECTION 4

Autres aspects de l'entreposage des produits congelés	Other aspects of frozen storage
A.H. BROWN (United Kingdom). Reliable refrigeration isn't expensive	141
<i>Une technique frigorifique fiable n'est pas coûteuse</i>	
H. MIKI, J. NISHIMOTO (Japan). Prediction of storage and thawing conditions by kinetic parameters on quality changes in fish muscles	145
<i>Prévision des conditions de conservation et de décongélation par les paramètres cinétiques des changements de qualité du muscle de poisson</i>	
W.H. EARL, D.H. ALLEN, L. KINDLEYSIDES (United Kingdom). Simulation modelling of frozen food distribution - retail operations and their effect on quality	153
<i>La représentation par modèles de simulation des opérations de distribution et de détail des produits alimentaires congelés</i>	

I.M. MACKIE, P. HOWGATE, A. CRAIG, W.M. LAIRD, A.H. RITCHIE (United Kingdom). Acceptability of frozen stored consumer fish products	161
<i>Acceptabilité du poisson congelé vendu au détail</i>	
J. NIELSEN, A. BRUUN, A. MADSEN (Denmark). Milk powders as cryoprotectants in frozen minced fish	169
<i>Les poudres de lait comme cryoprotecteurs des hachis de poisson congelé</i>	

SECTION 5

Procédés tendant à prolonger l'entreposage des produits réfrigérés	Possible methods of extending chilled storage life
G. HOBBS, F.J. LEY (United Kingdom). Radiation preservation of fish	177
<i>Conservation du poisson par irradiation</i>	
R.G. RICE, W.S. OTWELL, N. BLAKE, D.E. SWEAT, R.L. MARSCHALK, J.W. FARQUHAR (U.S.A.). Ozonized water and ice to preserve fresh fish grouper (<i>Mycoteroperca bonaci</i>)	187
<i>L'eau et la glace ozonisées pour la conservation des poissons frais : mérou (<i>Mycoteroperca bonaci</i>)</i>	
L.W. BAHRS (Fed. Rep. of Germany). Chilled storage of wet fish in modified atmospheres	195
<i>Entreposage frigorifique du poisson frais en atmosphère modifiée</i>	
G. BALDRATI, F. AMBROGGI, F. CASTELVETRI (Italy). Cold storage of sardines packaged in a modified atmosphere	199
<i>Entreposage frigorifique de sardines emballées en atmosphère modifiée</i>	
M.S. HENDRIE, D.C. CANN (United Kingdom). The relationship of temperature to the shelf life of vacuum packed marine fish - an interim report	205
<i>Relation entre la température et la durée de conservation de poisson emballé sous vide - rapport préliminaire</i>	
S. GOLA, M. ROSSI (Italy). Vacuum-packaging of fresh fish : effect of oxygen permeability of packaging material on the development of botulinal toxin	211
<i>Conditionnement sous vide de poisson frais : effet de la perméabilité à l'oxygène des matériaux de conditionnement sur la production de la toxine botulinique</i>	

SECTION 6

Etudes sur l'entreposage des poissons et autres produits de la mer réfrigérés	Chilled storage studies on fish and shellfish
M. MLAY (Tanzania), N. DIOUF (Senegal), Z. KARNICKI, C. LIMA DOS SANTOS (Italy). Storage life of chilled African fish species	219
<i>Durée de conservation par réfrigération d'espèces africaines de poisson</i>	
W.F.A. HORNER (United Kingdom), J. CASTRO REAL, D. REYNIER VALDEZ, E. ROCHA MILLER (Mexico). Some observations on the spoilage of tropically caught fish at different storage temperatures	227
<i>Quelques observations sur la détérioration des poissons tropicaux</i>	
G.C. FLETCHER, D.N. SCOTT, R.J. SEELYE (New Zealand). Shelf life of chilled orange roughy - research strategy for a new fishery	235
<i>Conservation en magasin de détail de <u>Hoplostethus atlanticus</u> réfrigéré - stratégie de recherche pour une nouvelle pêche</i>	
P.D. BAIRD, R. BENNETT, M. HAMILTON, J.G.M. SMITH, K.J. WHITTLE (United Kingdom). Consumer acceptability as the basis for determining the chilled shelf lives of smoked products from thawed herring and mackerel.	243
<i>Test de consommation comme base de la détermination de l'aptitude à l'entreposage par réfrigération des produits fumés, préparés avec des harengs et maquereaux décongelés</i>	

J.J. LICCIARDELLO, E.M. RAVESI, S.M. GEROW, D. D'ENTREMONT (U.S.A.). Storage characteristics of iced whole loligo squid <i>Caractéristiques de l'entreposage de calmar entier glacé</i>	249
---	-----

SECTION 7

Amélioration et contrôle de la distribution traditionnelle des poissons réfrigérés	Improving and monitoring traditional handling of chilled fish
L. GRAM (Denmark). Conductance measurement as a method for determination of the bacteriological and organoleptic quality of chilled fish <i>Mesure de la conductance pour déterminer la qualité bactériologique et organoleptique du poisson réfrigéré</i>	261
M. HANUSARDOTTIR, J. ZACHARIASEN, L. MOHR (Faroe Islands). Handling and storage of cod on board inshore boats <i>Manipulation et entreposage du cabillaud à bord de navires côtiers</i>	269
I. McDONALD, J. GRAHAM (United Kingdom). Variations in potential shelf life resulting from boxing at sea practice <i>Variations du potentiel d'aptitude à l'entreposage dû au procédé de mise en caisse à bord du bateau</i>	275
B.L. TINKER, J.W. SLAVIN, R.J. LEARSON, V.G. AMPOLA (U.S.A.). Evaluation of automated time-temperature monitoring system in measuring the freshness of chilled fish <i>Evaluation d'un système de surveillance automatique de la durée et de la température pour la mesure de la fraîcheur du poisson réfrigéré</i>	281
R.M. STOREY (United Kingdom). Time temperature function integration, its realisation and application to chilled fish <i>Intégration de la fonction temps température, sa réalisation et son application aux poissons réfrigérés</i>	293

SECTION 8

Présentation par affiches	Poster presentations
G.R. AMES, C.A. CURRAN (United Kingdom). Iced storage life of two fish species from Vanuatu <i>Durée de conservation dans de la glace de deux espèces de poisson de Vanuatu</i>	301
D.M. GIBSON (United Kingdom). "Malthus" and chilled fish storage <i>Le "Malthus" et l'entreposage du poisson réfrigéré</i>	307
P. BYKOWSKI, W. KOLODZIEJSKI (Poland). Chemical composition and quality of frozen roller-peeled krill meat <i>Composition chimique et qualité de chair de krill décortiqué au rouleau</i>	311
F. JIMENEZ-COLMENERO, M. TEJADA, A.J. BORDERIAS (Spain). Changes in protein functionality in fish muscle <i>Modifications des propriétés fonctionnelles des protéines du tissu musculaire de poisson</i>	319
P. NESVADBA (United Kingdom). Radiation heat transfer to products in refrigerated display cabinets <i>Transfert de chaleur par rayonnement aux produits dans les meubles de vente frigorifiques</i>	323

D.N. SCOTT, M.G. HOGG (New Zealand). Development and use of a taste panel for the determination of shelf life	331
<i>Formation et utilisation d'un jury de dégustation pour la détermination de la conservation du poisson en magasin de détail</i>	
R. HASTINGS, G. RODGER (United Kingdom). Differential scanning calorimetry of frozen cod : pH effects	337
<i>La calorimétrie à balayage différentiel du cabillaud congelé : effets du pH</i>	
B. JESSEN, N.C. JENSEN (Denmark). Prevention of black spot in retail-packed frozen Norway lobsters	343
<i>Prevention des tâches noires dans les langoustines congelées, en emballages de détail</i>	
SHANN-TZONG JIANG, CHING-YU TSAO, TUNG-CHING LEE (Taiwan). Prevention of browning discoloration and drip loss of frozen shucked oyster (<i>Crassostrea gigas</i>)	349
<i>Prévention contre le brunissement et l'oxydation des huîtres écaillées congelées</i>	
H.R. KHANDAKER (Bangladesh), M. HOLE, C. JUKES (United Kingdom). Packaging films for fatty fish preservation by freezing - relevance to Bangladesh.	355
<i>Pellicules d'emballage pour la conservation des poissons gras par congélation - applicabilité au Bangladesh</i>	
W.M. LAIRD, I.M. MACKIE (United Kingdom). The use of cyanogen bromide to study protein cross-linking during frozen storage of the flesh of cod (<i>Gadus morhua</i>)	361
<i>Utilisation du bromure de cyanogène pour l'étude des liaisons croisées des protéines de la chair de cabillaud (<i>Gadus morhua</i>) congelée au cours de son entreposage</i>	
P.A. PERIGREEN, J. JOSEPH, M.R. NAIR (India). Freezing and cold storage of Indian fish	369
<i>Congélation et entreposage du poisson aux Indes</i>	
P. REECE (United Kingdom). The fate of reduced nicotinamide adenine dinucleotide in minced flesh of cod (<i>Gadus morhua</i>) and its association with formaldehyde production during frozen storage	375
<i>Evolution de la NADH (Nicotinamide Adenine Dinucleotide réduite) dans du hachis de cabillaud (<i>Gadus morhua</i>) et son association avec la production de formaldéhyde au cours de l'entreposage à l'état congelé</i>	
J. WIGNALL (United Kingdom). Frozen fish in distribution in the United Kingdom	381
<i>La distribution du poisson congelé au Royaume-Uni</i>	
P. HOWGATE (United Kingdom). Bibliography of storage lives of wet and frozen fish	389
J. DAVIS (United Kingdom), B. LEPITRE (France). A leap forward in fresh fish marketing : the darfresh packaging concept	403
<i>Poisson frais : un saut dans l'innovation - le concept d'emballage darfresh.</i>	

TABLES RONDES - ROUND TABLES

K.J. WHITTLE (United Kingdom). Summary report on round table discussion of chilled storage	411
I.M. MACKIE (United Kingdom). Summary report on round table discussion on frozen storage	413
Round table discussions - Conclusions of the Organising Committee	415

FOREWORD

Marine products constitute a considerable resource for all nations, both industrialised and developing. In fact, these products contribute towards combatting famine and malnutrition and moreover they have an international exchange value.

Nevertheless, even today the biological cycle of marine animals has not been entirely mastered. Indeed, aquaculture and fish breeding are activities which are being developed extensively but with the majority of species it is not a question of full scale breeding as compared with animals for the butcher's shops or domestic animals: certain phases or reproduction or growth are not controlled. In another aspect, the economic importance of aquicultural production is small as compared with the value of conventional fish catches.

In other words, and even nowadays, the exploitation of marine food resources is closer to a harvesting activity than a production activity. This situation partly explains why the present output of food materials from the sea is low and, in consequence, why it is necessary to manage these resources more economically and give them better upgrading.

One way of increasing these resources is to extract more edible substances from marine biotopes: another way is to reduce losses.

In 1981, the International Institute of Refrigeration was invited to Boston to examine advances in the refrigerated treatment of fish. During that meeting particular stress was placed on increasing available food-stuffs by exploiting underutilised species.

In 1985, the Institute was invited to Aberdeen, another pole in the fishing world where more attentive examination was made of the means to provide longer storage and the better overall preservation qualities of fish and marine products.

All the papers presented at the Aberdeen meeting along with a substantial summary of the discussions are collated in this book which constitutes the 60th volume in the IIR series "Refrigeration science and technology".

This book is a valuable documentary source for all those interested in the refrigerated treatment of marine products. For those who already possess the Boston proceedings, this book constitutes, in addition, a complement as well as an updating of industrial knowledge and progress for those involved in the upgrading of fisheries.

AVANT PROPOS

Les produits de la mer constituent une ressource considérable, pour tous les pays, industrialisés et en développement. Ils contribuent, en effet, à lutter contre la faim et la malnutrition. Ils sont aussi une matière d'échange pour le trafic international.

Pourtant, encore aujourd'hui, le cycle biologique des animaux marins n'est pas entièrement maîtrisé. Certes, la pisciculture comme l'aquaculture sont des activités en plein développement. Mais, pour la plupart des espèces, il ne s'agit pas d'un véritable élevage comparable à celui des animaux de boucherie ou des animaux domestiques : certaines phases de la reproduction ou de la croissance ne sont pas contrôlées. Par ailleurs, l'importance économique des productions aquacoles est minime par comparaison avec la valeur de la pêche traditionnelle.

En d'autres termes, encore de nos jours, l'exploitation des ressources alimentaires marines est plus voisine d'une activité de collecte que d'une activité de production. Cette situation explique, en partie, pourquoi le rendement actuel en matières alimentaires des océans est faible et en conséquence pourquoi il convient de gérer cette ressource avec plus d'économie et de mieux la valoriser.

Un des moyens d'accroître la ressource est d'extraire plus de substances consommables des biotopes marins ; un autre moyen est de réduire les pertes.

En 1981, l'Institut International du Froid avait été invité à Boston pour examiner les progrès en matière de traitement par le froid des poissons. Au cours de cette réunion, il avait été mis en particulier l'accent sur l'accroissement des disponibilités alimentaires par l'exploitation des espèces sous-utilisées.

En 1985, l'Institut a été invité à Aberdeen, un autre pôle pour le monde de la pêche, où ont été plus attentivement examinés les moyens de préserver plus longtemps et mieux l'ensemble des qualités des poissons et produits de la mer.

Toutes les communications présentées à Aberdeen et un résumé substantiel des discussions ont été réunis dans le présent ouvrage qui est le 60^e tome de la collection de l'IIF : « Science et technique du froid ».

Cet ouvrage est une source documentaire remarquable pour tous ceux qui s'intéressent au traitement par le froid des produits de la mer. Il constitue de surcroît, pour ceux qui disposent du compte rendu de Boston, un complément ainsi qu'une actualisation des connaissances et des progrès industriels pour ce qui a trait à la valorisation de la pêche.

ALLOCUTIONS D'OUVERTURE
OPENING SPEECHES

Welcoming speech by Professor Jack Connell,
Director of Torry Research Station, Aberdeen

My task as stand-in for the Minister of State is to welcome you all to Aberdeen and to this meeting. The programme for the meeting gives the temperature in Aberdeen as 9°C. I am told the actual temperature today is 19°C, so I hope the warmth of the climate we have arranged for you is matched by the warmth of the hospitality you enjoy during your stay. I am not sure whether it is Aberdeen or the subject of this meeting that has attracted you here, but whatever the reason I must express my personal pleasure on seeing so many participants, several of whom are old friends or acquaintances. The excellent response the organisers have had from all over the world, and what I think is the high standard of papers to be presented, augurs well for the discussions over the next 3 days.

The subject of our meeting is "Storage Lives of Chilled and Frozen Fish and Fish Products". The two main reasons for holding the meeting at this time are, first, the growing importance of marketing better quality fish and fish products and, secondly, the need to market fish in new forms, the behaviour of which may not be adequately known. Of course, fish quality and new products from fish have been preoccupations of many of us here for a very long time, but I have a strong impression through reading and general contacts that they are matters that have assumed even greater importance recently. In respect to both matters, the fish industries require clear information about how long it is possible to store a wide range of refrigerated fish before quality falls below certain predetermined limits. Such information allows the design on rational principles of economic storage and distribution systems. Also, the demands by consumers for more information about storage lives is giving rise to a great extension of open date marking by which is meant a clear statement on the label of the date beyond which the product should not be sold or stored. Public Authorities also need advice about storage lives so that any regulations about temperatures or periods of storage that they formulate and enforce can be practical and realistic. Subsidiary reasons for this meeting are, first, the fact that research on storage lives could lead to useful ways of improving preservation and, secondly, the possibility that energy saving and greater efficiency of operations could result if storage temperatures can be increased.

A good deal has been written and spoken about storage lives, but much of what has emerged is unsatisfactory or out of date. For example, various definitions of storage life exist but there is no consensus on which should be used in particular cases. There is confusion about methods for measuring storage life, and inconsistencies and errors in the interpretation of the results. In some cases the data are inapplicable to modern products packed in modern materials. Not enough is known about the effects, if any, of fluctuating temperatures and of other variables.

We should not be concerned by the fact that the meeting deals exclusively with fish. Undoubtedly, similar questions affect other food commodities. The justification for our exclusivity is that fish does have special and rather more acute problems of preservation and, as we all know, fish has usually led the way in refrigeration. Thus, although at first sight the subject of this meeting seems rather narrow and sectorial, in

fact, as I have just indicated, it holds a wealth of important and interesting details for those who have to deal with refrigerated foods. The purpose of this meeting is to present new decisive facts and some clear thinking on the topic of storage lives, and on the topics surrounding it. To achieve this purpose the organisers have provided us with a systematic framework within which to operate. We have, if I may say so, an excellent group of experts here today. Given these advantages, I have every hope that we can achieve something very useful.

Address by Dr. A. Gac
Director of the International Institute of Refrigeration

It is always an honour as well as a great deal of satisfaction for the Director of the International Institute of Refrigeration to participate in a Commission meeting. As defined in Article 2 of the International Agreement which governs the Institute, the fundamental mission of the IIR is to promote and disseminate research results and industrial advances relating to refrigeration and all its applications.

To accomplish this mission, the IIR possesses only two principal sources of information. The first is the exploitation of scientific and technical journals, reviews and books published all over the world; in this pursuit, the IIR receives more than 300 periodicals, the articles of which are referenced in the IIR Bulletin which gives a brief summary of the contents in English and French. As from next year, the Institute will be putting its computerised database, FRIGINTER, into operation and it will contain all the documentation published in the Bulletin, summaries included, since 1981. In addition, the documentary richness of the Institute is exceptional in that the first issue of the Bulletin dates back to 1909 and its library to day carries nearly 4000 books.

The second source of information comprises Commission meetings and International Congresses. During these events experts from any country, whether or not it is a member of the Institute, discuss their observations and present their results, thus contributing to making people acquainted with the advances realised at all stages, from fundamental research laboratories or research centres and testing stations, up to industrial realisations and every day practices. However, in order to make these meetings profitable it is necessary to publish reliable and good quality proceedings. In this respect, the Institute is very attentive in making sure that the papers presented are of high level and original, that they are prepared in a concise but clear manner and that they are backed up by a summary of the discussions resulting from the presentation of the papers.

In the particular case of the Aberdeen meeting, I would like to add that the invitation from the United Kingdom was a specially attractive one because it opened the doors of the Torry Research Station to research workers and professionals who know that Torry is one of the most famous centres in the world for research on fish and sea products.

In the name of the International Institute of Refrigeration and on behalf of those present here, I sincerely thank the United Kingdom as well as the authorities of the Torry Research Station. I would also wish to extend our profound compliments to Mr. Graham and his team of co-workers in having planned and organised this meeting.

Allocution par le Dr. A. Gac
Directeur de l'Institut International du Froid

C'est toujours un honneur et aussi une très réelle satisfaction pour le Directeur de l'Institut International du Froid de prendre part à une réunion de Commission. En effet, la mission fondamentale de l'IIF, telle qu'elle est définie à l'Article 2 de la Convention Internationale qui le régit, est de promouvoir et diffuser les acquisitions de la recherche et les progrès de l'industrie concernant le froid et toutes ses applications.

Pour accomplir cette mission, l'IIF ne dispose que de 2 sources principales d'information. La première est l'exploitation des journaux, revues et ouvrages scientifiques et techniques publiés dans le monde entier; l'IIF reçoit ainsi plus de 300 périodiques, dont les articles sont référencés dans le Bulletin de l'IIF, avec un bref sommaire en français et en anglais de leur contenu. Dès l'année prochaine l'IIF va mettre en service sa base informatisée, sous le nom de FRIGINTER. FRIGINTER est constituée par la totalité de la documentation publiée dans le Bulletin, avec les analyses, depuis 1981. En outre, la richesse documentaire de l'IIF est exceptionnelle, puisque le premier numéro du Bulletin remonte à 1909 et que sa bibliothèque compte près de 4000 ouvrages.

La seconde source d'information est constituée par les réunions de Commissions et Congrès Internationaux. Au cours de ces manifestations, les experts de tout pays, membre ou non de l'Institut, débattent de leurs observations, confrontent leurs résultats et contribuent ainsi à faire connaître les progrès réalisés à tous les stades, depuis les laboratoires de recherche fondamentale ou les centres d'études et stations d'essais, jusqu'aux réalisations industrielles et à la pratique quotidienne. Mais, pour que ces réunions soient profitables, il convient d'en publier un compte rendu fidèle et de bonne qualité. Aussi, l'Institut est très attentif à ce que les communications présentées soient originales et de haut niveau, qu'elles soient rédigées de façon concise mais claire et qu'elles soient complétées par le résumé des discussions qu'elles ont suscitées.

Mais dans le cas particulier de la présente réunion, je dois ajouter que l'invitation reçue du Royaume-Uni était spécialement attractive. Elle ouvrait en effet aux chercheurs et professionnels les portes de la Torry Research Station, l'un des centres de recherche les plus fameux au monde sur les poissons et produits de la mer.

Au nom de l'Institut International du Froid et au nom des participants ici présents, je remercie très sincèrement le Royaume-Uni ainsi que les dirigeants de la Torry Research Station et j'adresse les compliments les plus chaleureux à M. Graham et à tous ses collaborateurs.

LISTE DES PARTICIPANTS - LIST OF PARTICIPANTS

AFRIQUE DU SUD - SOUTH AFRICA

BOTHA W.G., Cape Town

ALLEMAGNE (Rép. Féd. d') - GERMANY (Fed. Rep. of)

FOX J., Bremerhaven
REHBEIN H., Hamburg

SCHREIBER W., Hamburg

ALLEMANDE (Rép. Dém.) - GERMAN DEMOCRATIC REPUBLIC

MANN W., Berlin

AUSTRALIE - AUSTRALIA

BREMNER A., Hobart

CANADA

CORMIER A., Moncton
LECLERC L., Quebec

MERRITT J.H., Halifax
RODMAN W.K., Halifax

CHILI - CHILE

GALECIO-ASAYA C., Osorno

DANEMARK - DENMARK

BORRESEN T., Lyngby
GRAM L., Lyngby
HERBORG L., Copenhagen
HUSS H.H., Lyngby
JESSEN B., Lyngby

JUL M., Copenhagen
KNØCHEL S., Lyngby
NIELSEN J., Lyngby
SKARE S., Hirtshals

ESPAGNE - SPAIN

BORDERIAS A.J., Madrid
DIEZ F., Vizcaya
MORAL A., Madrid
PEREZ-VILLAREAL B., Vizcaya

POZO R., Vizcaya
SALINAS I., Vizcaya
TEJADA M., Madrid

ETATS-UNIS - UNITED STATES

FARQUHAR J., Washington
JOHNSON C.E., Madison
LEARSON R.J., Gloucester

REID D.S., Davis
RICE R.G., Ashton
SLAVIN J.W., Annandale

FEROE (Iles) - FAEROE (Islands)

HANUSARDOTTIR (Mme), Torshavn

FRANCE

BECEL P., Boulogne-sur-Mer
ESTOCQ M., Aubigny en Laonnois

HAN-CHING L., Nantes
LEPITRE B., Epernon

GUYANE - GUYANA

GORDON R.M., Georgetown

INDE - INDIA

GOPAKUMAR K., Cochin

IRLANDE - IRELAND

FISHER G.W., Cork

BUTLER T.C., Dublin

ISLANDE - ICELAND

ARASON S., Reykjavik

MÖLLER A.B., Reykjavik

ITALIE - ITALY

BALDRATI G., Parma

LESTINI L., Rome

JAPON - JAPAN

FUJITA K., Kawasaki
KOZIMA T.T., Tokyo

MIKI H., Kagoshima
NISHIMOTO J., Kagoshima

NORVEGE - NORWAY

BERG N., Oslo
BLOKHUS H., Bergen
CHEN S., Trondheim
EGEBERG T., Trondheim
EIDE O., Tromsø
JEBSEN J.W., Bergen

MALNES D.W., Tromsø
MAGNUSSEN O.M., Trondheim
RASMUSSEN T., Stavenger
SKAARA T., Stavenger
SLINNING K.E., Stavenger

NOUVELLE-ZELANDE - NEW ZEALAND

SCOTT D.N., Auckland

PAPOUASIE-NOUVELLE GUINEE - PAPUA NEW GUINEA

OLIVERA R., Lae

PAYS-BAS - *NETHERLANDS*

ALOFS M.J.M., Rotterdam
DA PONTE D.J.B., Wageningen

HOUWING H., Ijmuiden

POLOGNE - *POLAND*

BYKOWSKI P.J., Gdynia
MALKOWSKI J., Gdynia

OZAROWSKI M., Gdynia
WENSKI L., Szczecin

PORTUGAL

DE MATOS L.C., Lisbon

NUNES M.L.B., Lisbon

ROYAUME-UNI - *UNITED KINGDOM*

AINSWORTH P., Manchester
AITKEN A., Aberdeen
ALDERSON R., Inverness
AMES G.R., London
ARTHEY D., Chipping Campden
BULL K., London
BENNETT R., Aberdeen
BROUGHTON I.R., Liverpool
BROWN A., Glasgow
BROWN M., Sharnbrook
CLARK A., Doncaster
CONNELL J.J., Aberdeen
COWIE W., Aberdeen
CROSSLAND J.S., Grimsby
EARL W.H., Stirling
GIBSON D.M., Aberdeen
GRAHAM J., Aberdeen
HARDY R., Aberdeen
HARRISON D., Edinburgh
HASTINGS R.J., Aberdeen
HEAP R.D., Cambridge
HENDRIE M.S., Aberdeen
HEWITT M.R., Aberdeen
HOBBS G., Aberdeen
HODSON G., London
HOLE M., Grimsby
HORLICK M., Carshalton
HORNER W.F.A., Grimsby
HOWGATE P.F., Aberdeen
JAMES S., Bristol

JUKES C., Grimsby
KENT R., London
LAIRD W.M., Aberdeen
LEY F.J., Swindon
McDONALD I.J., Aberdeen
McINTOSH J.C., Waltham Cross
MACKIE I.M., Aberdeen
MADDEN R.H., Belfast
MARSHALL D.W., Newcastle
MILLER R., London
MILLS A., London
MUNN S., London
NESVADBA P., Aberdeen
NEWMAN M.J., Grimsby
PARK J.R., London
PARSONS A., Leeds
PEARMAN P., Waltham Cross
POULTER R.G., London
REECE P., Aberdeen
ROBINSON H., Waltham Cross
SANDERSON-WALKER M., Walton-on-Thames
STOREY R.M., Hull
TAYLOR J., Chipping Campden
URCH M., Epsom
VICKERS D., Hull
WHITTLE K.J., Aberdeen
WIGNALL J., Aberdeen
WILDING P., Sharnbrook
WILLIS S., Grimsby
YOUNG K., Aberdeen

SUEDE - *SWEDEN*

ANDERSEN E.M., Bjuv
LILJEMARK A., Göteborg
LINGNERT H., Göteborg
LUNDBERG A., Bjuv

OLOFSSON M., Bjuv
SMEDBERG B., Bjuv
ÅKESSON G., Göteborg

SUISSE - *SWITZERLAND*

BINDSCHEDLER O., Vevey

TAIWAN

JIANG S.T., Keelung

TCHECOSLOVAQUIE - CZECHOSLOVAKIA

HOREJSI J., Prague

STOKLASA J., Prague

O.A.A. - F.A.O.

KARNICKI Z.S., Rome (Italie)

I.I.F. - I.I.R.

GAC A., Directeur

EXPOSÉ INTRODUCTIF
INTRODUCTORY SPEECH

CHILLING AND FREEZING FISHERY PRODUCTS; CHANGES IN VIEWS AND USAGES

MOGENS JUL

Department of Food Preservation
The Royal Veterinary and Agricultural University
Howitzvej 13, DK-2000 Frederiksberg (Denmark)

1. FRESHNESS TESTS

Objective tests

For many years, fishery products technology seemed somewhat misled by the notion held by many scientists in that field, namely that before meaningful experiments could be carried out with regard to various fish preservation methods, one needed some method for the objective measurements of freshness. Fishery products technology research in most countries invested heavily in experimentation in this area. In a way, this was not productive. It was quite clear that any person experienced in the fishery industry or, as a matter of fact, experienced as a consumer in the consumption of fish, would have a pretty good idea of the required degree of freshness for fishery products and would be able to determine with acceptable accuracy whether one preservation method was to be preferred over another, etc. Further, it seemed that the opposite attitude, i.e. that such a decision could only be made on a basis of an objective test, was in a way a deception. Sometimes, i.e. at high temperatures, the breakdown of fresh fish is mainly due to microorganisms, around zero-temperatures, it is due to enzymatic protein breakdown, and for frozen fish at say at -20°C , it is due mainly to oxidation of lipids or protein denaturation. In all cases, a limit for consumers' acceptance would exist, which could easily be determined subjectively, but the chemical or physical descriptors would be completely different. If one focussed on those, as has often been the tendency, erroneous conclusions could be drawn.

Eventually, the search for an objective test for freshness was largely given up, possibly in frustration and sometimes even with insufficient considerations for real life conditions. It is quite clear that an objective method had - and has not yet - been devised, which would serve scientific purposes except as a diagnostic tool. On the other hand, when it comes to official quality control, it is indeed very useful to have an objective method. Thus, it is much easier for a health inspector or fishery officer to make decisions on the basis of an instrumental or chemical test instead of using purely personal judgments which will always be open to criticism and at times to litigation. Thus, the use of the trimethylamine test for freshness of fresh fish in Canada probably was a useful step. One need to realize, however, that such tests are arbitrary. Lightly spoiled products may escape the test, but it is unlikely that a sample with a high trimethylamine content would not be spoiled.

There is another situation where an objective test, even with its shortcomings, is useful. This is in the relationship between a supplier and a buyer. Thus, General Foods, Inc., at one time purchased fish on the then existing fish pier in Boston harbour on the basis of an alkali/acid titration test. The method was eventually abolished because it was cumbersome and sampling was difficult. However, when used, it was found useful in preventing the otherwise very unsatisfactory disputes between trawler skippers and fish purchasers. Interestingly, a total volatile nitrogen (TVN) test has today been introduced in contracts between trash fish suppliers and Danish fish meal factories (Jensen, 1984). This has led to very significant increases in the quality of the landings, resulting in a better yield for the factories and, environmentally, a considerably step forward. The use of an organoleptic test would have led to many disputes. While the TVN-test is not perfect, it serves the purpose.

Subjective tests

However, in science the interest in objective freshness tests dwindled, and interest was focussed on the sensory test. Here, the mere fact that a few experienced people might judge one sample acceptable and another not, conflicted with the scientists' strict requirements for objectivity. Therefore, sensory tests were developed with an increasingly sophisticated degree of objectivity. The number of panellists is normally not less than 10, the panels are always given samples anonymously, and results are, of course, statistically treated. The procedure is quite laborious and costly.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

At times, one may question the correct use of sensory tests, however. They were devised in an effort to make the subjective evaluation of freshness as well-defined and objective as possible. It is a question whether in doing this to a very considerable degree one has not almost lost sight of the purpose of the test. This is practically always to decide the limit for acceptability of a product. However, the conditions under which tests are carried out are so extreme that they, of course, have little relationship with the way food is evaluated in real life, and one does not really know how the results of the panel tests can be interpreted. As a matter of fact, in a recent EEC collaborative study in the poultry sector under the EEC Standing Committee for Agricultural Research, SCAR, it was revealed that none of the participating organizers of test panels could give even a qualitative indication of the correlation between the findings in sensoric tests and consumers acceptance.

Consumers preference

In so far as consumers preference is concerned, the picture is far less clear than normally assumed. It is well-known that populations close to the sea may require a degree of freshness that actually is rejected by some inland populations. Similarly, what is found in the frozen fish trade today would by many be characterized as rancid, by some even as highly rancid. Producers and distributors are aware of this, but they are also aware of the fact that if they receive complaints about a product, it is generally because it contains bones. Obviously, the presence of bones is easily determined. Therefore, it is much more likely that complaint will be lodged against this defect. Rancidity is a matter of taste, and consumers may dislike it without quite knowing why and without complaining about it. However, the degree to which rancidity is tolerated in the frozen fish trade is surprising, and probably suggests a reevaluation of our concept of quality of fishery products. Some assume that what is most like a very fresh raw material is what will be preferred by the consumer. However, marketing experience and the study of consumers behaviour do not support this view.

2. HANDLING ABOARD

Preservation on board

Traditionally, fishery products from anything but near coastal waters have been preserved aboard by packing in ice, either in pounds or boxes in the hulls. Actually, the widespread use of ice has been one of the saving graces in fishery products preservation. This is because ice is easily made available, it can be purchased in the necessary quantities, it is capable of delivering a large amount of refrigeration in a short time, and it maintains a very definite temperature, i.e. -0.5 to 0°C where sea water is present.

However, packing in ice, whether in boxes or pounds, is labour intensive and must be replaced by other methods. Refrigerated sea water (RSW) was labour saving and an acceptable, but not ideal successor. It requires complicated pumping and filtering systems; it is highly costly and impractical to install on board a compressor of sufficient capacity to chill large catches quickly. An inherent defect of the system is that one no longer has a guarantee that 0°C will be maintained. As a matter of fact, we have a guarantee that it will not, because, if it were, the condenser would most likely be covered by ice.

The system may be replaced by chilled sea water (CSW) systems, i.e. systems where sufficient ice is taken along on a fishing voyage and eventually mixed first with water and then with fish when catching occurs. Both systems have the advantage that the fish can be dumped directly into the holds.

The CSW system also does not guarantee the maintenance of 0°C because mixing ice and sea water is not always as thorough as could be desired. For one thing, the ice tends to stay frozen together in the bottom of the tank. Today, the system is being supplemented with tubes through which compressed air is sent up from the bottom through the tanks to ensure proper mixing of fish, water and ice, the so-called champagne method. In principle this should be the ideal solution. However, there is now a tendency for the ice to keep floating in the upper part of the tank, so 0°C is still not always achieved. Also, insufficient ice may be a problem. In Denmark, attempts are being made to require temperature recording in the tanks. Some fishing boat owners have protested against this additional expenditure, although, when the total expenditure in equipping a fishing vessel is considered, this is but a nominal cost.

3. CHILLED FISH

Retail distribution of fresh products

Unavoidable and actually very desirable changes have affected not only handling fish on board but also its handling on land. When it comes to fresh fish, fish was traditionally sold

from slabs where they were packed with ice, again with a pretty good guarantee that the fish was maintained at 0°C. Also, that method of preservation requires daily if not hourly inspection, etc. This, at least, could guarantee a certain degree of discipline with regard to the freshness of the product. Such fish slabs with ice are seen even in very modern supermarkets in most of Europe. However, they do constitute a rather awkward element; thus, in Denmark, for instance, supermarkets seem unwilling to accept the messiness and smell of iced fish, and they seek other means of distributing fresh fish.

One solution seemed to be packing the fish in modified atmosphere in a plastic film container. However, this never was any success, the packages are bulky and the fact that they are air filled makes it difficult to maintain a low temperature of the product. One Danish food chain has given up this system for fresh fish.

It seems that vacuum packing fresh fish has a better chance of being a suitable method for fish distribution. Interestingly enough the film does not have to be oxygen impermeable because the bacterial flora on the fish surface metabolises the small amounts of oxygen penetrating the package, so no rancidity can be found (Huss, 1983). The method seems to be gaining in acceptance at least for fatty species (Hansen, 1982). Yet, one Danish food chain recently discontinued marketing fresh fish this way.

Whether packed in modified atmosphere or in vacuum, packaged products will be sold from refrigerated cabinets. Here again, there is no guarantee that temperature will be as low as 0°C, or rather, there is almost a guarantee that it will be higher, because if the temperature in some area is as low as 0°C, it will be considerably higher in other areas, or some fish would actually freeze in the cabinet, which is not acceptable.

Thus, interestingly enough, progress in chilled food handling and distribution has for the fishery industry actually led to the products being distributed at a somewhat higher temperature than before, i.e. 2-4°C, where the traditional method was packing in ice at 0°C. The traditional fish-monger still exists and may have a connoisseur clientele but the trade seems to be disappearing. The modern food trade has yet to devise a proper system, including the supply organization, for the distribution of fresh fish or the trade may eventually disappear.

Deep chilling

One may wonder whether there in the fresh fish trade might be a niche for deep chilled (superchilled) fish. Experience with poultry in the USA was that there is a very considerable consumer preference for fresh poultry over frozen poultry. However, it was difficult to distribute regular fresh poultry over long distances. Therefore, deep chilling was introduced, i.e. crust freezing the product and maintaining it at about -1 to -2°C throughout the storage and distribution periods and at a nominal +2°C in the retail stores. This process is now in very widespread use in the US broiler chicken industry.

Interestingly, the process was in use in fisheries in Denmark in the 1930's where an export of cod fillets was based on the fillets being packed in 1 kg packages which were crust frozen at -7°C in so-called hardening rooms prior to being shipped in wooden boxes insulated with excelsior and with layers of ice separate from the packages of fish.

However, even deep chilling could result in a display temperature of the product which would be above the traditional 0°C, simply because of the limitation of the refrigerated display cabinets.

One question to be answered is if there would be a market today for such a product. Consumers behaviour does not make this too likely, it seems.

TTT-studies for chilled fishery products

It is well-known that for frozen products many studies have concentrated on determining the time-temperature-tolerance, so-called TTT-studies. Today, there is a similar need for determining the time-temperature-tolerance of chilled foods. In the fishery industry this seems particularly important because one can no longer rely on the maintenance of the magic figure, storage temperature of 0°C.

One very critical part of any such TTT-studies is, however, what was mentioned above. It is very hard to say what the limit for acceptability of fishery products really is. It almost seems that any person with a thorough knowledge of fishery products technology is by that very fact disqualified from making any judgments in this regard. Other means will have to be

found, but none has yet been clearly identified. It may be that any such method have to be based on the assumption that consumers behaviour is predictable, and this assumption may prove to be incorrect.

PPP-studies for chilled fishery products

It is also well-known that the time-temperature-tolerance of frozen products is highly dependent on the so-called PPP-factors, i.e. product, process and packaging. These factors may have to be included also in studies on chilled foods.

The **product** factor is well-known. It may depend on fishing area, sexual cycle, fishery method, treatment of the fish on board, etc., etc.

The **process** factor is also likely to be highly important. If one reviews the display of chilled fish products in today's supermarkets, one will notice that this fairly infrequently contains fresh fish. It is more common to find lightly smoked or lightly salted products, etc. These products are gaining in acceptance, presumably because of the fact that they lend themselves much better than unprocessed fresh fish to modern retail distribution because of a considerably longer shelf life in the chilled state.

The **packaging** factors were referred to already above.

Distribution chain survey for chilled fish

There seems to be a need also for surveys of temperature conditions prevailing in the chilled fishery products chain, as suggested in table 1, and comparable to the ones shown in table 2.

TABLE 1

Estimated maximum storage times and temperatures for the 75% of meat products in the Danish chilled food chain experiencing the **least** unfavourable conditions. (After Soerensen, 1985).

	Average time days	Average temperature °C
Producer and wholesale level	6 to 19	3 to 8
Retail cabinet	2 to 19	2 to 10

Table 1 shows preliminary data from a detailed study on meat products in Denmark, obtained by Soerensen (1984). In reviewing temperatures in retail cabinets, cf. Magnussen (1984), one might wonder if vertical chilled cabinets with glass doors like those frequently used for dairy products seems to offer better temperature control than horizontal cabinets. However, the trade seems to disfavour them. Both time-temperature surveys and investigation of PPP-effects could be part of the COST 91 bis Project, dealing with chilled foods.

Boegh-Soerensen and Zeuthen (1984) and Gram and Pedersen (1984) have demonstrated that one may actually use time-temperature-tolerance data in conjunction with time-temperature survey data in order to get, by some simple calculations, a rough estimate of the products quality, e.g. as measured by remaining shelf-life, at the point of purchase and after the product has passed a normal refrigerated distribution chain. The procedure would be similar to what has long been suggested in frozen food contexts.

4. FREEZING

Freezing has become the preservation method **par excellence** in the fishery industry. Conditions have changed very considerably since frozen fish were frozen according to the Ottesen method, in brine, and sold often thawed as fresh fish. Today, all sorts of combined, reformed or almost restructured products are sold as fish fingers, fish sticks, etc., etc.

PPP-factors

When reviewing fishery products in frozen food cabinets in a supermarket today, one notices how widespread uses are made of the various PPP-factors. Thus products are often covered with a gravy or they may be breaded; both are measures which yield considerable

protection against desiccation and rancidity. Some products are vacuum packaged or glazed with the same purpose (Hansen, 1983).

Christensen and Jessen (1984) showed that contrary to what is the case for cured meat products, cured fish does not exhibit reverse stability even if in oxygen permeable packages at freezer temperatures.

One often meets cases where, surprisingly, no use is made of the PPP-factors. Thus, one often finds small flat fish fillets labelled as individually packaged. They are actually layer packaged with a piece of impregnated paper zig-zagged between the fillets leaving the ends and sides open to oxidation. In testing the product, one will find very definite signs of rancidity after some months of storage. However, such occurrences do not seem to affect sales.

It seems quite obvious that there is a need for more time-temperature-tolerance studies in the field of frozen fishery products. This may be particularly important because fish is increasingly double frozen. It seems simply necessary in a modern industrialized society to maintain factories working on an all year basis. This is contrary to tradition in the fishery industry since practically all fisheries are seasonal. The only option then is freezing the raw material for processing later. It is well-known that this can be done without any noticeable loss of quality or shelf life but also that it requires careful planning and especially carefully controlled raw materials handling, thawing and further treatment. Also, the extended fishing trips for many species require at sea freezing for processing ashore. It is hoped that authorities will accept frozen raw material as a basis for further processing, refreezing or sale in the thawed, "fresh", state.

Time-temperature surveys

In so far as time-temperature surveys for the frozen food chain is concerned, considerable data are available from the experiments carried out under the so-called COST 91 Programme. Some results of that study are given by Zeuthen *et al.* (1984), and some are summarized in table 2. Here, the results from UK were only temporary at the time of the conclusion of the COST 91 Programme.

TABLE 2

Maximum temperatures and times experienced by the 75% of frozen products exposed to the **least** unfavourable conditions (Zeuthen *et al.*, 1984).

	UK		FRG		DK	
	Several		Fish sticks		Ground meat	
	°C	Weeks	°C	Weeks	°C	Weeks
Central cold store	-22	-	-24	7	-23	16
Distributors cold store	-18	-	-24			
Retail store	-11	-	-12	8	-12	5
Total time in chain		17				

Data such as these may be useful tools in estimating the quality of frozen fish at the point of sale or at the point of consumption after they have been kept in a home freezer. However, here again the difficult factor is the determination of the limit for acceptability among consumers.

5. COLLABORATIVE EEC-STUDIES

It may be useful to make some additional references to various collaborative EEC studies. Above, reference was made to the COST 91 study already concluded on frozen foods, described by Zeuthen *et al.* (1984). Reference was also made to work by Soerensen (1985). This is advance results of the present EEC collaboration, COST 91 bis, which concerns itself with chilled foods.

The so-called EEC Agro-Food Programme, also referred to above, is also of interest. It presently has collaborative programmes under way in the field of determining the relationship between laboratory tests, objective as well as subjective, to consumers' reactions and a study of consumers' perception of quality in foods. As was mentioned above, these are probably some

of the most puzzling aspects of fishery products technology today, and the collaborative programme may for that reason be of interest for fishery research also.

Many may question the quality of fresh or frozen fish as it appears in the trade today, yet consumption is increasing in spite of relative large price increase; thus, in Denmark, consumption rose from 14 kg per cap. some years ago to 20 kg today. Therefore, also, it would be interesting to get some clearer ideas of what consumers' reactions and expectations actually are.

REFERENCES

1. BOEGH-SOERENSEN, L. and ZEUTHEN, P. (1984). The validity of the TTT-concept on the shelf lives of chilled cured meat products. Paper 5:5. In: Proceedings of the 30th European Meeting of Meat Research Workers. Meat Research Institute, Langford.
2. CHRISTENSEN, A.T. and JESSEN, B. (1984). TTT-studies of cured fish products. In: Zeuthen, P.; Cheftel, J.C.; Eriksson, C.; Jul, M.; Leniger, H.; Linko, P. (eds.). Thermal processing and quality of foods. Elsevier Applied Science Publishers, London.
3. GRAM, L. and PEDERSEN, P. (1984). Tid-temperatur-tolerans hypotesen. Afdelingen for Ievnedsmiddelkonservering, Den kgl. Veterinaer- og Landbohøjskole, København. Thesis.
4. HANSEN, P. (1982). Detailpakning af fersk fed fisk. Fiskeriministeriets Forsøgs-laboratorium, Lyngby. Reprint.
5. HANSEN, P. (1983). Frysning og frostlagring af fed fisk. Fiskeriministeriets Forsøgs-laboratorium, Lyngby. Reprint.
6. HUSS, H.H. (1983). Fersk fisk, kvalitet og holdbarhed. Fiskeriministeriets Forsøgs-laboratorium. Lyngby.
7. JENSEN, P.F. (1984). Personal communication.
8. MAGNUSSEN, O.M. (1984). In: IIR 16th International Congress of Refrigeration, Commission 2. IIR, Paris.
9. SOERENSEN, I.-M.J. (1985). Personal communication.
10. ZEUTHEN, P.; CHEFTEL, J.C.; ERIKSSON, C.; JUL, M.; LENIGER, H.; LINKO, P.; VARELA, G. and VOS, G. (eds., 1984). Thermal processing and quality of foods. Elsevier Applied Science Publishers, London.

REFRIGERATION ET CONGELATION DES PRODUITS DE LA PECHE; EVOLUTIONS DANS LA CONCEPTION ET LA PRATIQUE

RESUME : Beaucoup de tests objectifs ont été proposés pour déterminer le degré de fraîcheur du poisson. On considère souvent que ces tests ont une validité limitée pour les études scientifiques, mais qu'ils sont pratiques pour le contrôle de qualité et les transactions commerciales. Les tests sensoriels peuvent sembler plus satisfaisants mais il est difficile de les corréler avec les seuils d'acceptabilité des consommateurs.

La manutention du poisson abandonne de plus en plus le glacage qui nécessite un travail pénible. Cependant, aucune des nombreuses méthodes de refroidissement par immersion ne peut garantir une température de 0°C, c'est à dire celle de la glace fondante.

Le commerce de détail aussi abandonne le glacage comme il est solissant et entraîne de mauvaises odeurs. Il en résulte que aujourd'hui le poisson est conservé et distribué aux températures plus élevées, 2-6°C, que dans le passé. Actuellement, le poisson, frais ou réfrigéré, n'est plus adapté aux pratiques commerciales et la distribution s'intéresse de plus en plus aux produits légèrement fumés ou saumurés.

La congélation est la méthode principale de conservation du poisson comme matière première ou produit fini. On exploite souvent un accroissement de la durée de vie résultant d'une particularité dans l'emballage ou le traitement.

A la fois pour les poissons réfrigérés et congelés il serait nécessaire de déterminer les couples temps/température tout au long de la chaîne de distribution aussi que la durée de vie des produits en fonction de la méthode de préparation, de l'emballage, etc.

SECTION I

QU'ENTEND-ON PAR APTITUDE À L'ENTREPOSAGE?

WHAT IS SHELF LIFE?

LITERATURE REPORTING OF SHELF LIFE DATA. WHAT DOES IT ALL MEAN?

R. J. LEARSON

NMFS, Gloucester Laboratory, Gloucester, Mass, USA

J. J. LICCIARDELLO

NMFS, Gloucester Laboratory, Gloucester, Mass, USA

I. INTRODUCTION

The issues of fishery product quality and quality improvement continue to be the major subjects of attention and contention in the world of fishery technology. Research technologists are continuing investigations on methods of quality extension and producers of fishery products are testing new methods for upgrading quality in order to meet the "new found" demand for high quality fishery products by consumers.

In 1983, in cooperation with the Refrigeration Research Foundation (Bethesda, MD) the National Marine Fisheries Service, Northeast Fisheries Center Utilization Division in Gloucester, MA carried out a literature search on reported shelf life values of frozen seafoods. This literature search was conducted in support of a proposed research project aimed at ascertaining 1) the relative costs of maintaining cold storage facilities for fishery products at temperatures lower than standard commercial conditions and 2) the relative benefits of product quality enhancement. The following communication represents an analysis of reported shelf life data in the scientific literature, and a critique of the methods used to determine shelf life and the manner in which the data are reported.

2. LITERATURE SEARCH

The literature search was primarily executed in a similar manner to a previous survey manuscript by J. P. Lane /1/ in 1964. Using access to computerized data bases and traditional library searching techniques, publications were selected on the basis of title and the appropriateness of the subject matter. Species selected were Atlantic and Pacific groundfish, salmon and herring. Only those publications which reported specific values for shelf life at specific storage temperatures were selected. The data as reported were transcribed and formatted into a tabular form showing the species, storage temperature, product form, special conditions (packaging, etc.), storage life and a reference number relating to the publication. Table 1 represents the data format used.

3. ANALYSIS OF REPORTED SHELF LIFE DATA

In order to determine the average values and relative variance of reported shelf life data, storage life values at specific temperatures for selected species were plotted in graphical form. Figure 1 shows the values reported for frozen Atlantic cod fillets and blocks at various frozen temperatures and Figure 2 shows a similar data set for haddock. For simplification, temperature values were plotted to the nearest whole fahrenheit degree and both shelf life and temperature values were averaged when a range was reported (e.g., 6-8 months = 28 weeks). Figure 3 presents trend lines for the average shelf life values as reported versus storage temperature for nine commercially important species.

4. DISCUSSION

In perusing the literature for shelf life values several problems became immediately evident. Depending upon whom you read or whom you believe, reported values range widely. For example, in Figure 1, various authors have reported the frozen shelf life of cod stored at 0°F to be anywhere from 15 to 45 weeks. In Figure 2 the range of shelf life values for haddock at 0°F range from 24 to 72 weeks. When reading the literature one can see obvious differences among investigators in methodology. Some researchers are using raw evaluations to estimate shelf life, while others employ cooked evaluations. A variety of hedonic scales are being used and it is difficult to determine from the literature if they are objective or subjective scales, and often there is no indication of the expertise of the sensory panel. In reading the literature one often gets the impression that the investigator himself/herself made an individual decision as to

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

the sensory quality of the product.

Most investigators use chemical objective tests such as DMA or rancidity measurements to correlate with sensory analysis; however, other researchers assess shelf life strictly on the basis of chemical indices without any sensory evaluation. Another problem is the use of chemical tests that don't relate to the objective, e.g., DMA in frozen herring.

Other problem areas noted relate to experimental design. Experiments are often reported in the literature which indicate that little or no thought went into certain important variables such as initial fish quality, seasonality, geographic location, fishing method, and proper sample sizes. Finally, the majority of investigators report only one value relating to the end of shelf life, which assumedly is the point where the product quality becomes unacceptable. This generates a considerable amount of confusion to producers, buyers and consumers of fishery products who may be interested in high quality seafoods as opposed to intermediate or low quality product. Investigators should be attempting to also report intermediate values where the product quality passes from good to fair or from Grade A to Grade B.

In some cases we as fishery technologists are actually doing a disservice to our constituency. We very neatly report that by using this new packaging material, new process, or additive, etc. the shelf life of the product is "doubled" or "increased by --- %." What we fail to report is that often times the extension of shelf life occurs in the intermediate or fair quality range and not in the range of high quality. Producers of fishery products making capital investments relating to quality improvement can well be misled by the typical data now appearing in the literature.

5. CONCLUSIONS AND RECOMMENDATIONS

It is clear from the scientific literature that fishery technologists have no standardized procedures for estimating shelf life which leads to wide variance in reported values. This deficiency usually leads to poor conclusions and recommendations which can be misleading to users of the data. The following is recommended:

1. A manual for quality/shelf life studies should be developed, along the lines proposed by Lima Dos Santos et al /2/, recommending standardized sensory testing procedures for seafood products.
2. The manual should include standardized chemical objective tests, with detailed procedures and discussions on what the tests actually measure, when they should be used, and how they correlate with sensory analysis.
3. The world community of scientists active in fishery technology should cooperatively develop standardized collaboratively studied procedures under the auspices of FAO, IIR and the AOAC to insure uniformity of results and quality assurance of analytical results.

REFERENCES

1. LANE, J. P. Time-temperature tolerance of frozen seafoods. I. Review of some of the recent literature on the storage life of frozen fishery products. Food Technology (1964) Vol. 18(7), 1100.
2. LIMA DOS SANTOS, C. A. M., JAMES, D. and TEUTSCHER, F. Guidelines for chilled fish storage experiments. FAO Fish. Tech. Pap. No. 210, (1981) 22p.

LA LITTÉRATURE SUR LA CONSERVATION EN MAGASIN. QUELLE EN EST LA VALEUR ?

RESUME: Les auteurs passent en revue les renseignements publiés sur la conservation commerciale des produits de la pêche congelés provenant de plusieurs espèces et ils les confrontent. Les renseignements sur la fin de la durée de conservation ont été regroupés dans des tableaux et des graphiques pour présenter les durées publiées par différents chercheurs. Les résultats montrent de grandes différences de durée de conservation pour des espèces semblables à des températures semblables. Cela signifie qu'une normalisation serait nécessaire pour la mesure et l'indication dans les publications de l'aptitude à la conservation en magasin.

TABLE I
TIME-TEMPERATURE TOLERANCE DATA

Species	Storage temperature	Product form	Remarks	Storage life (months)	Reference
Pacific hake <u>Merluccius productus</u> (Pacific whiting)	0 F	Portions	Unglazed Ice glazed	0.5 2.5	25
	-20 F	Portions	Glazed Antioxidants + packaging	6 6	
	-15 F	Fillet Block Minced block	Vacuum packed or glazed	12 6	15
	0 F	Filletlets		3.25	23
Argentine hake <u>Merluccius hubbsi</u>	0 F	Fillet block	4 day iced fish	1	18
	-9 F	Filletlets		3	20
	0 F	Blanched breaded sticks	Control Na erythorbate treated Saberized Saberized + erythorbate (sticks were prepared from 4 month old frozen treated or untreated blocks at 0 F)	2.5 6 7.5 11	26
	0 F	Blocks (fillet)	Control Na erythorbate treated Saberized Saberized + erythorbate	9.5 13.5 15 17	
	-22 F	Blocks (fillet)	(all above samples packed in 4 mil poly bags)	17	
Chilean hake <u>Merluccius gayi</u>	5 F	IQF fillets	Glazed or packed in impermeable film	5	
	5 F	Fillet blocks	1 day fish 7 day fish	6 4-5	17

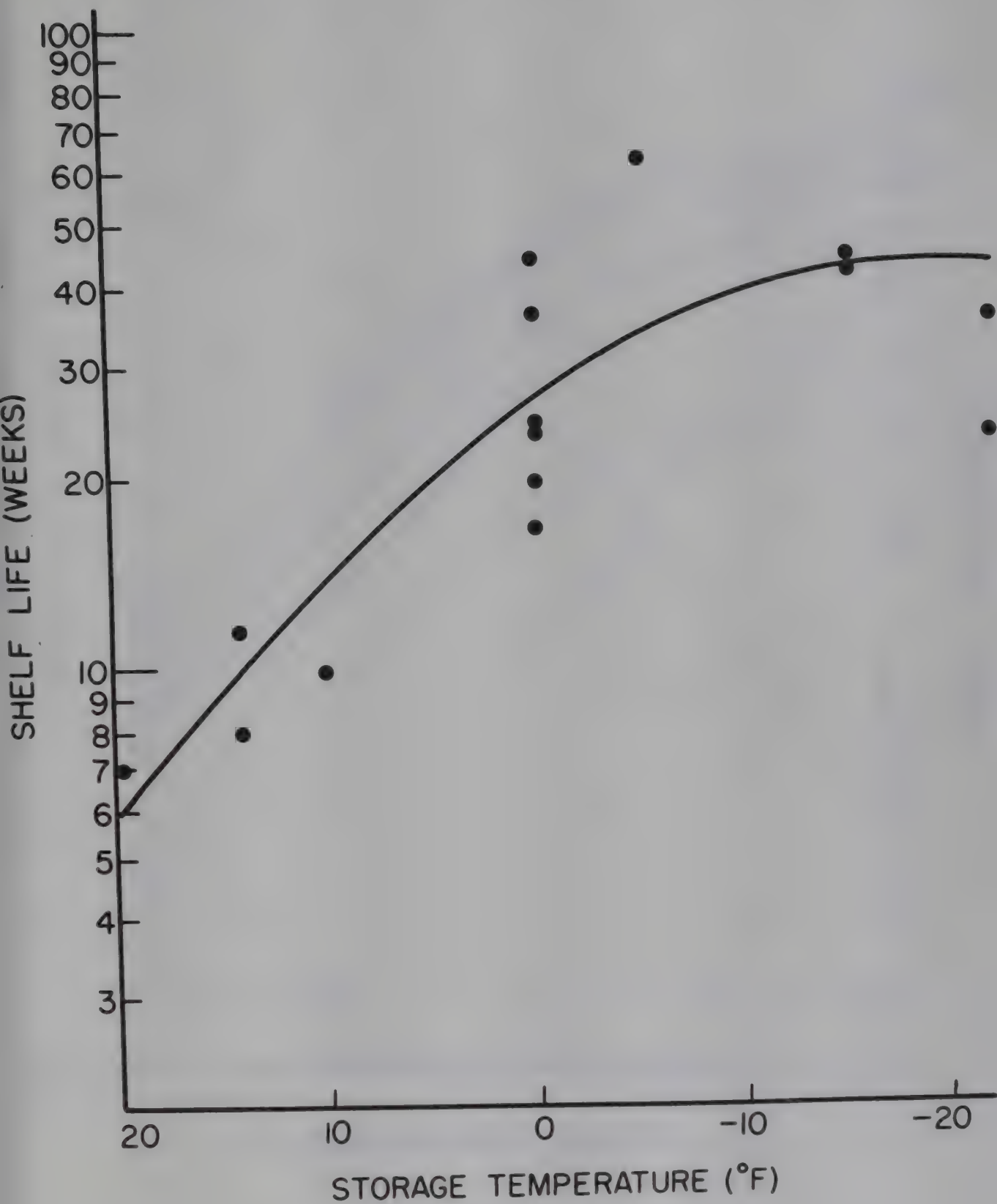


Fig. 1 - Storage Life of Frozen Atlantic Cod Based on
Literature Reported Values

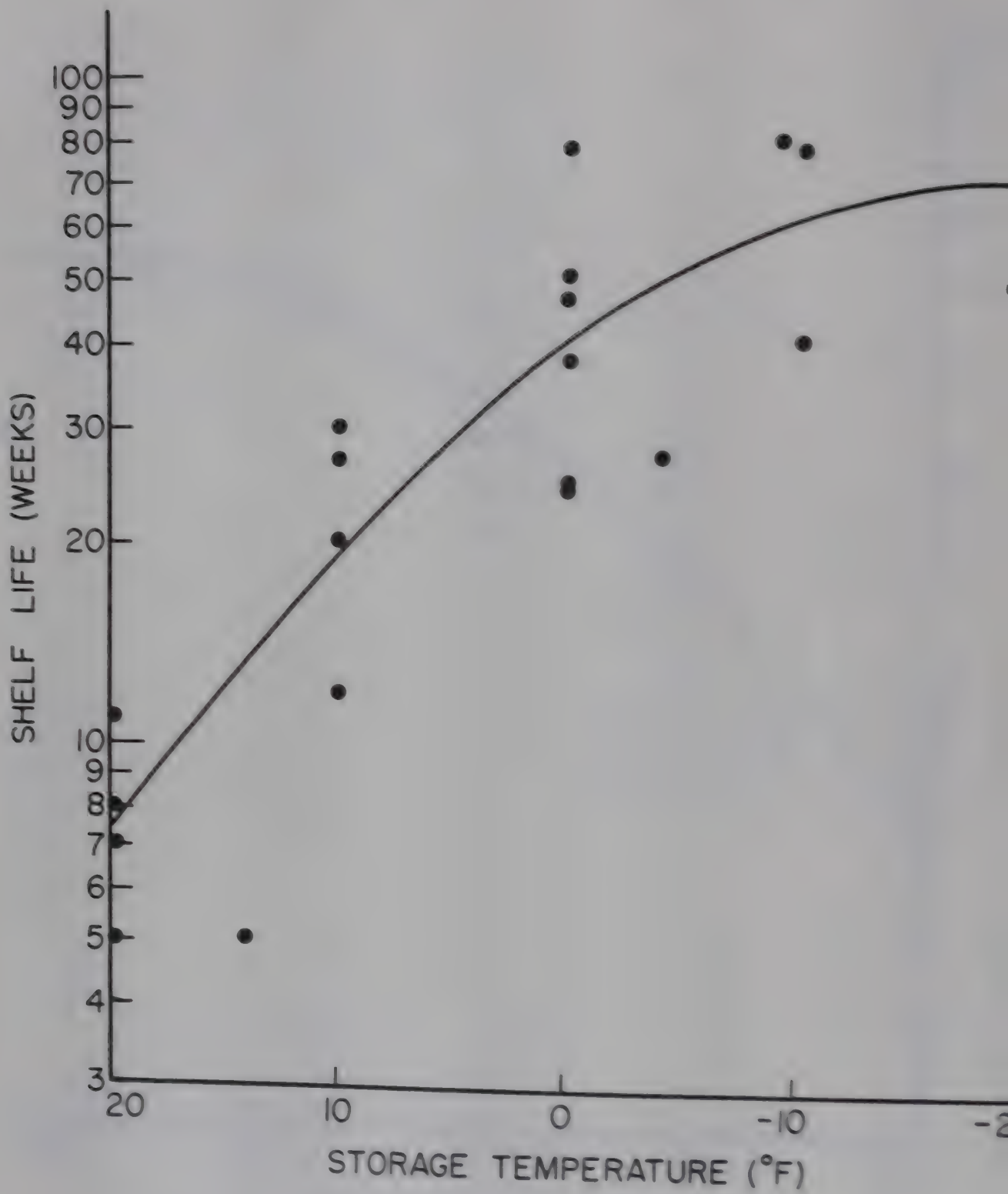


Fig. 2 - Storage Life of Frozen Haddock Based on Literature Reported Values

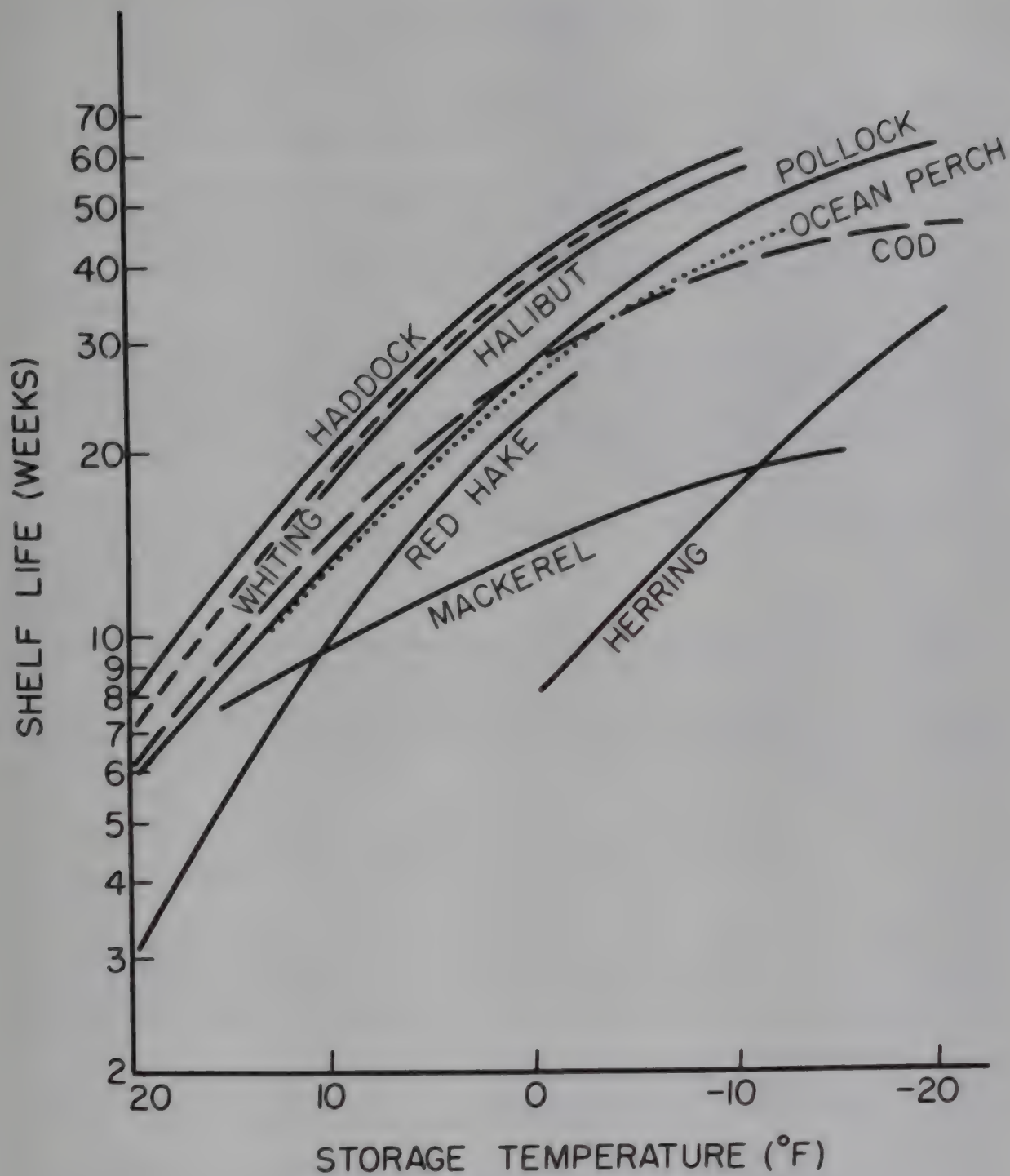


Fig. 3 - Average Shelf Life Values for Selected
Finfish Based on Literature Reported Values

DISCUSSION

A. BREMNER (Australia) - In the collaborative studies you are doing with the U.S. Institute of Refrigeration what tests will you be using to determine quality ?

R.J. LEARSON - We will be using sensory analysis using expert panels and also appropriate chemical tests including DMA in cod and ammonia in shrimp.

J.J. CONNELL (UK) - In your answer to the previous question you referred to methodology but did not say how you were proposing to define shelf-life. Do you have any views on 'What is shelf-life' ?

R.J. LEARSON - That is a key question. We define shelf-life as the end of acceptability. The question then is: 'to whom'? A trained panel will set a level of acceptability different to that of a consumer group. No one knows what the resulting relationship is and it may well be that it will be different for different consumer groups. For example, a consumer group from Boston as opposed to one from Kansas City. Under the Mid Atlantic Fisheries Development Foundation we have formed a committee to investigate this particular problem.

M. SANDERSON WALKER (UK) - Will you be using fluctuating or stable temperatures in your studies ?

R.J. LEARSON - Stable.

R. BENNETT (UK) - In your inspection of the discrepancies of shelf life reported in the literature, did you form any opinion of the reasons for the wide variations ?

R.J. LEARSON - Primarily, it appeared that many problems were associated with the sensory testing that was used to establish the end of shelf life. It was obvious from the literature that some investigations did not use experienced panels and often used inappropriate test methods.

R.G. POULTER (UK) - You make reference in the paper to FAO guidelines for chilled fish storage. This FAO document was prepared as the result of work done in developing countries on tropical fish species. Would you like to comment on the possibility of the wider use of these guidelines ?

R.J. LEARSON - I was just referring to the FAO release as an example of a set of guidelines. What I'm suggesting is a larger publication which would spell out appropriate sensory and chemical tests for different species and clearly show both the advantages and limitations when using the different tests with different species. This would promote greater consistency in the literature.

M. JUL (Denmark) - Are you sure that you really want standardised methods ? Shelf life is a very complex thing. Don't you think we stand a better chance of throwing light on this issue if laboratories use their own methods?

R.J. LEARSON - I am not saying that every laboratory should use the same method. What I'm proposing is some sort of manual which will describe appropriate tests and methods of reporting so that they can be understood and interpreted by both users and other scientists.

SHELF-LIFE - QUALITY:
A QUESTION OF RELATIVITY

LARS HERBORG
Fish Inspection Service
Ministry of Fisheries, Copenhagen (Denmark)

Introduction. Shelf-life and quality are often talked about as if they can be measured as easily as length and weight.

This conference deals with the matter in great detail and a thorough discussion is very much needed.

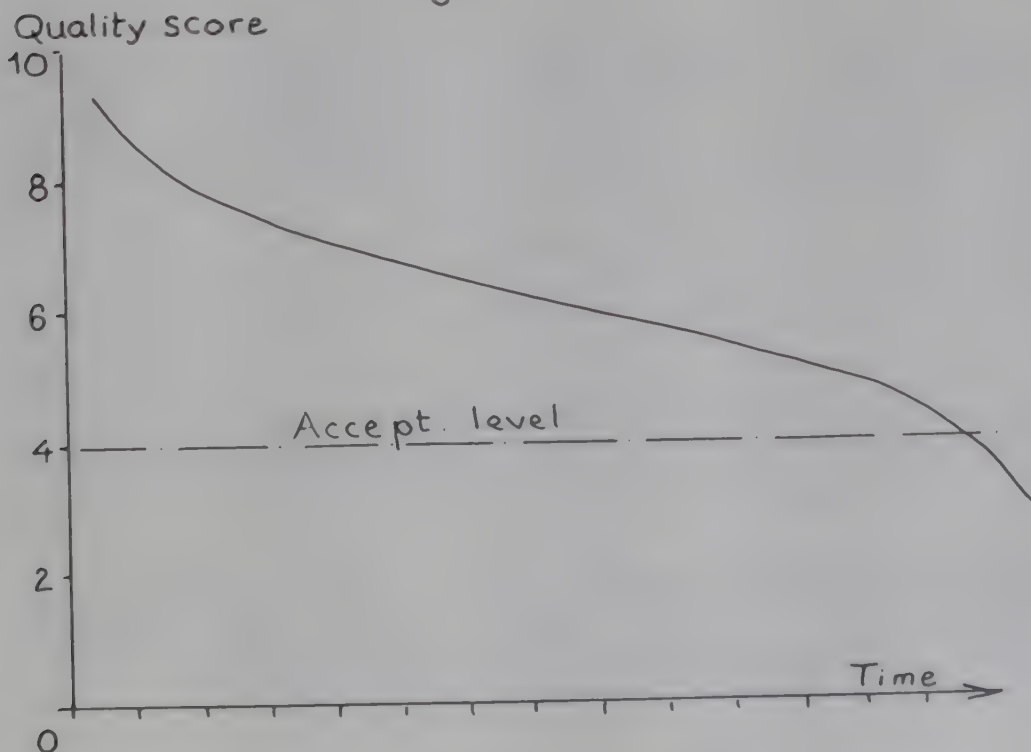
Terms like "time of minimum durability" is introduced by the EEC directive on labelling of foodstuffs, and defined as the time during which the foodstuff retains its specific properties when properly stored - at least the shelf-life must be somewhat longer, but what is the point of reference for quality, and what is the relation between expert panel acceptance and consumer acceptance?

With no prejudice to what will be demonstrated during the conference some problems related to the shelf-life/quality complex will be dealt with in the following.

Fresh fish

It is often anticipated, that the live fish represents the maximum quality. This is a truth with limitations, however, after the fish is dead the degradation begins which usually means a deterioration of the quality. Let this be illustrated by the classical relation between quality and storage time as determined by sensoric assessment as shown in Fig. 1.

Fig 1.



This S-shaped curve is well known and is the basis for decisions on the improvement of the technology of handling, processing and distribution of fish, fresh as well as frozen. The sensoric assessment is still the key, regardless the correlation to numerous objective methods. The results are the Bible. But how are the results obtained?

Generally speaking the results are obtained from sensoric assessments by panels trained to give the "right" answers, i.e. the panelists do set their reference points of quality to fit the "house" scoring scale for quality. The acceptability level is constant.

The panellist must learn to suppress individual likes/dislikes and try to function as an objective instrument - learn that the sting of guts which is negative for one fish may be a characteristic for another, the taste of flounder is good for flounder, but only fair for plaice and so on. The quality is associated with the specific properties of the individual fish - it is relative.

Adding to this, samples to be assessed sensorically are prepared in different ways. It is surprising that there seem to be sort of agreement between laboratories on storage experiments - at least for white fish.

By improving the technology of handling the catch, i.e. cooling the fish better, gutting the fish, exclusion of oxygen etc. the level of acceptability is reached after a longer and longer period of time. This means that the fishing boats can stay longer at sea, or the fish can reach destinations at longer distance than before. The time before the level of acceptability is crossed is extended and so is the shelf-life, but does that mean that the consumer is getting the same quality as before - but from older fish? Maybe, but not necessarily. Apparently the trader of fresh fish is looking for the highest quality fish and this means fish less than one week on ice, preferably one or two days. Some traders believe that the fish is losing the gloss if iced all over and prefer fish laid on ice - they do not care whether the fish will keep 2 weeks if properly iced, because they sell the fish before 2 days anyway. Those traders are not the less prosperous.

Consumers of fresh fish seem to have a rather stable point of reference for quality - relatively speaking because quality might mean different things depending on the geographical situation - continental/coastal. Typically the consumer of fresh fish wants to inspect the fish when bought or rely on the trader's recommendation - the pre-pack is not very attractive in this respect. Consumers of fresh fish might be more interested in the art of cooking.

But what happens to the other fish?

Frozen fish

In a recommended international code of practice for frozen fish it is written as a general consideration that fish intended for freezing should be of the highest possible quality. It is explained that freezing and frozen storage cannot improve the quality of fish, at best the condition of the fish before freezing can be maintained. For this reason it is essential that the raw material is as fresh as possible.

This is a reasonable statement of intent, however, observations have been made on the fact, that the initial quality of the raw materials is levelled out by freezing and frozen storage, e.g. as described by Connell & Howgate in 1968 /1/ and Herborg & Jakobsen in 1977 /2/ for fish of the cod family as illustrated in Fig. 2.

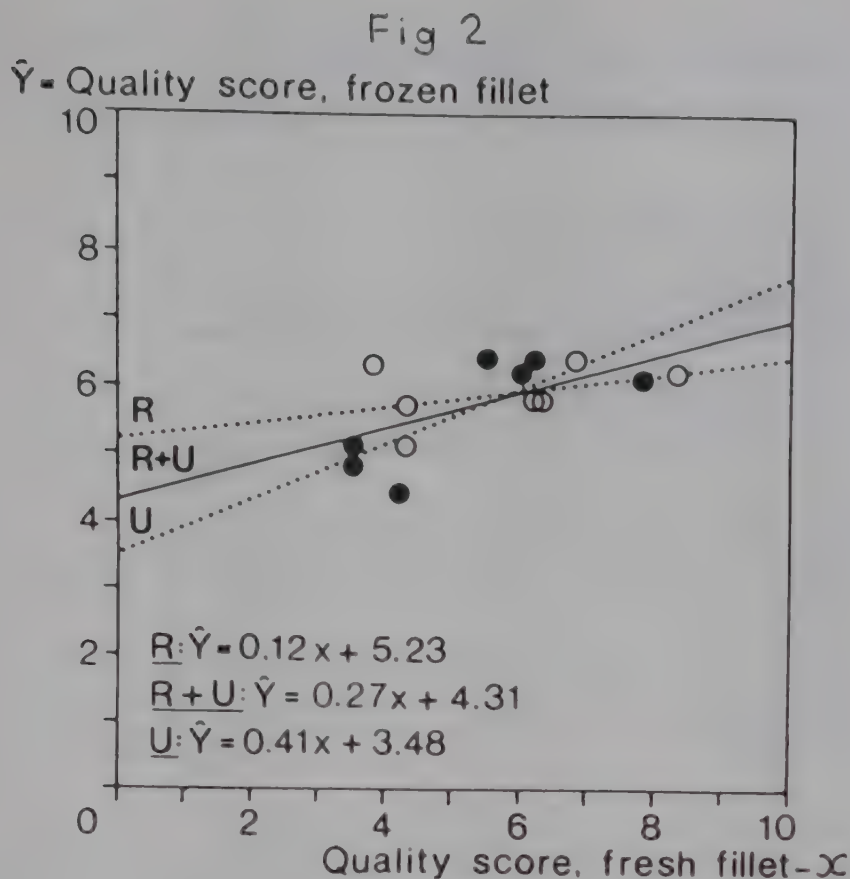
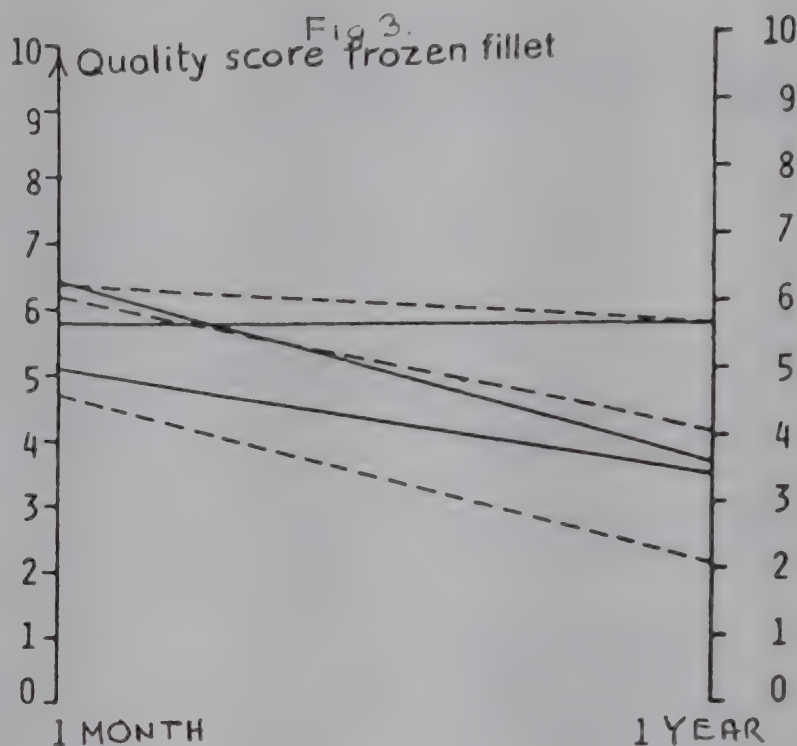


Fig. 2. Quality of cooked fresh and thawed whiting fillets after frozen storage in 1 month. The regression lines represent whiting gutted on catch (R) and whiting gutted on landing (U) as well as total material (R + U).

Fillets were cut from fish iced for 2-14 days and the frozen fillets were packed in 16½ lbs. blocks and platefrozen and stored under commercial conditions for one month - one year at -20°C before assessment. It should be noted that some of the members of the assessment panel were managers from the industry and they seem to be weak when approaching the level of unacceptability. Nevertheless the freezing process seem to change some of the specific sensoric properties of the fresh fish and the highest quality fresh fish suffers most. The freshness is lost from the freshest fish and the staleness of the low quality fish is dispersed and/or masked by other flavours developing during frozen storage. Relatively speaking the quality of low quality raw materials seems upgraded after some time on frozen storage.

During storage over a one year period under commercial conditions some samples seem to retain their quality, others are deteriorating as illustrated by Fig. 3.



The number of samples is too small to draw any conclusions as to why the quality will deteriorate, but the samples are produced under commercial conditions and reflects in a way what could happen in practice, and also indicates how difficult it is to apply the EEC definition on minimum durability on frozen products.

It must be pointed out that the quality/keeping time complex might look different, if the frozen fish product appears in a different shape such as single frozen fillets or breaded products or otherwise treated products. The result of the sensoric assessment will also be different if carried out according to the final use of the product, e.g. according to the serving suggestions.

Therefore, the importance of the absolute quality of the fish in the products might be minor. Quality could also be related to the end use of the product.

The shelf-life should be related to data from storage experiments of the typical products under commercial conditions. The quality level is not only the responsibility of the manufacturing or distributing companies, but could as well be a matter of public interest, particularly if the market mechanisms are giving a slowly sliding overall quality, because the competition is on price alone.

The fish inspector's role ?

The difficulties related to assessment of quality and the corresponding shelf-life of frozen fish products has been mentioned and the impression could be, that this matter is too complex to be dealt with legally and therefore not a matter for the public fish inspector. The problem should be left between buyer and seller - be a matter of sales strategy - at least as long as the consumer is not exposed to any health risk. This could be true, but some facts should be considered, particularly for frozen fish and fish products.

For health reasons consumption of fish should be encouraged. Since

consumers of fresh fish normally are fans of fish and conscious about quality, relatively speaking, anyway, it might be wise to encourage the group of frozen fish consumers not only through price and convenience but also through quality. Price and convenience are very important in a stressing time with dwindling cash resources for food, but quality should be given a high priority too.

Although quality of frozen fish is a difficult matter to deal with because of the poor relation to the quality of the raw materials and the strong relation to the end products, there seem to be a demand for some guidelines for the trade for the estimation of the decline in quality and the corresponding shelf-lives of products.

There are too many examples of mal practice during distribution which might be due to ignorance. Frozen fish products do not last for ever, and some products are more sensitive to quality loss during storage than others.

E.g. it is evident that single frozen flat fish fillets in bulk will keep shorter than breaded or block packed or vacuum packed fillets, however, the products are often sold with the same shelf-life.

Although the poor products will not be of any hazard to the health they might be unfit for human consumption due to rancidity, desiccation or denaturation, and the public fish inspector could play an important role by looking after the product quality in the retail shops.

Legal action could be difficult in some countries due to the lack of objective methods for measuring quality and due to the relativity of quality in general. Legal action could also be difficult if the quality in fact is accepted by the consumers, who buy the product again and again - their reference point is set to that standard, and provided the slip of standard is sufficiently slow the consumers will adjust their reference point accordingly - standard comes before quality - however, there must be a limit to the tolerance, and it is a matter for the public fish inspector to set the limit if the trade have difficulties in finding the line.

This can be done by following the products through all their history with due respect to the conditions during which the products are distributed and sold.

REFERENCES

1. J.J. CONNELL & P.F. HOWGATE: Sensory and objective measurements of the quality of frozen stored cod of different initial freshness. J.Sci.Fd.Agric. 1968, Vol. 19, p. 342-354.
2. L. HERBORG & G. JAKOBSEN: Utilization of by-catches of whiting and haddock, gutted on landing, for the production of frozen fillet blocks. Unpublished report, 1977. Technological Laboratory Danish Ministry of Fisheries.

APTITUDE A LA CONSERVATION - QUALITE : UNE QUESTION DE RELATIVITE

RESUME : Bien que la qualité dépende de la demande des consommateurs, de la nature du produit et de son emballage ainsi que des conditions de distribution et de vente, et bien que la qualité des poissons congelés soit, dans certains cas, faiblement fonction de leur qualité initiale, il convient de prendre en considération l'amélioration de la qualité générale, en particulier des poissons congelés et d'estimer l'aptitude à la conservation avec le souci de mieux répondre à la nécessité d'améliorer la qualité. Ceci devant être fait en coopération avec les pêcheries, l'industrie du poisson, la distribution, la recherche et l'administration chargée du contrôle du poisson.

APPROACHES TO THE DEFINITION AND MEASUREMENT OF THE STORAGE LIFE OF CHILLED AND FROZEN FISH: A BRIEF REVIEW

P. Howgate
MAFF, Torry Research Station, Aberdeen (United Kingdom)

1. INTRODUCTION

Over the last 40 years or so there has been a considerable amount of research into the changes that occur during the storage of fish and fish products in both the wet and frozen states and much of this work has been oriented towards measuring the storage lives of the products. Any reviewer of the topic is faced with a very large body of literature on the subject consisting of primary sources, that is accounts of investigations to determine storage lives, and more general articles summarising and commenting on storage lives. A computer aided search of Food Science and Technology Abstracts using as keywords storage life, shelf life and fish recovered almost 90 references of which about half came from primary sources. Such a search of course can only recover articles scanned by the abstracting service over the period of currency of the data bank - in this case since 1969 - and having the keywords in the title or abstract. A more extensive search by conventional methods has so far produced over 150 references in which the authors have investigated changes in stored fish and made a pronouncement on the storage life of the product. These references came from research journals or reports of investigations published directly by research institutes. Even so I would not claim to have found all the relevant references since I have examined predominantly writings in English and I have not assiduously pursued reports from research institutes.

In spite of this large volume of writings I was disappointed to find that very few authors define what they mean by 'storage life' or 'shelf life' or whatever term they use to denote the limit to the storage period. Nor do they describe the properties of the fish at this limit. The usual procedure has been to have a small panel of experts or near expert assessors to assess the fish on a scale of value judgements such as like/dislike, good quality/bad quality, excellent/poor. Some point on this scale, sometimes but not always anchored by a term like just acceptable, is designated as a limit and the time during the storage trial when this value is reached then becomes, by definition, the storage life. This is a circular definition and it is not possible for the reader to know what criteria the assessors used to assess the quality of the fish at this point.

In this review I will examine some definitions of storage life that have appeared in the literature and discuss them in relation to methods for measuring it. The review is brief and the bibliography is selective rather than comprehensive. A more complete bibliography of values of storage lives appears as a poster contribution to this Conference. Lima dos Santos /1/ has published fairly recently a review of the storage life of chill stored fish and Lane /2/ in 1964 one on frozen stored fish. The IIR itself has issued summary tables of the storage lives of chilled /3/ and frozen /4/ fish.

2. THE BASIC CONCEPT OF A LIMIT TO THE TIME FISH PRODUCTS CAN BE STORED

The properties, and particularly the organoleptic properties, of a food or food product are not stable following harvesting or manufacture but, allowing for those changes accompanying maturation, change in a way which reduces the quality of the product. Quality here means the aggregate of properties which influence the food's acceptability to the consumer or user. From this principle arises the concept of a limit to the length of time a particular food product can be held under the stated conditions of storage and still remain of the quality required.

This limited period has been designated by several names by people in the food industry including those working with fish. By far the most common in the literature reviewed by me is 'storage life'; 'shelf life' is used in less than 10% of the references. 'Keeping time' is very rare, appearing in only 3 or 4 papers. In a few papers the authors have used more than one term. In this review I have joined the majority and generally used 'storage life' though in places where the discussion requires it I have used an alternative.

Outside the context of fish technology I would like to draw attention to the European Community (EC) Directive on food labelling /5/ which includes requirements for the date stamping of foods. It uses the term 'minimum durability' ('durabilité minimale' in the French language version) to designate the storage period to which the date stamp applies. The same term appears in the corresponding British Regulations /6/ which derive from this Directive. (Participants of this Conference may wish to note that the British Regulations specifically exclude frozen foods from the requirement to carry a date of minimum durability; the EC Directive does not.) Though all these terms express the principles behind the concept of a limit to the period for which fish may be stored they tend to be used in different areas of activity in the food industry and food control and have slightly different meanings. I would like to differentiate 3 areas of interest: food science and technology, food marketing, and food legislation and consumer protection. The areas overlap but designation in this way may assist debate on how storage life should be defined and measured.

3. DEFINITION OF STORAGE LIFE AND OF CRITERIA FOR DECIDING THE END OF STORAGE LIFE

The term 'storage life' judging from its use in technical literature seems to express best the ideas of fish technologists - and probably food technologists generally since it is used in the IIR Recommendations /3, 4/. Those for chilled foods /3/, para 1. 12 defines 'expected or practical storage life' as 'the greatest length of time for which the bulk of produce may be stored either with maximum commercially acceptable loss of quality and nutritive value or with maximum acceptable wastage by spoilage'. The English is inelegant and the definition clumsy. Anything which is wasted by spoilage must be beyond an acceptable loss of quality. The loss of nutritive value is important for some food products but has not been suggested as a limiting factor for fish products.

The IIR Recommendations for frozen fish has 2 definitions. High Quality Life (HQL) is the minimum time during storage of the product at which a change can be detected in a sensory difference test. This is very much a food scientists' definition and this approach to finding an end point was developed for studying factors, particularly temperature, which influence rates of change during storage of frozen foods /7/. It was not intended for measuring storage life and its use for this purpose has been criticised for several reasons /8, 9, 10/ not least because this approach leads to storage times which are too short to be of any commercial significance. My view is that the definition should not be used though the term HQL could usefully be retained but with another definition.

The second type of storage life in the IIR 'Red' book /4/ is the Practical Storage Life (PSL) defined as 'the period of frozen storage after freezing of an initially high quality product during which the organoleptic quality remains suitable for consumption or for the process intended'. The definition becomes general when the words 'frozen' and 'after freezing' are removed. I am sure that the general definition would be recognised by the great majority of fish technologists as expressing what they are trying to measure when investigating the storage lives of fish products, wet or frozen. Though this IIR definition is rarely acknowledged as such it is evident from an examination of the literature on measurement of storage life of fish products that the criteria which have been used for deciding on the limit to storage life could, almost without exception, be fitted within it. Almost invariably sensory methods have been used to measure changes during storage and suitability (or more usually acceptability) for consumption has been a frequent criterion of quality. Less frequently technological quality, the required quality for further processing, has been the criterion. Though these criteria have been, implicitly or explicitly, applied in storage trials for determining shelf life they have been applied differently by different workers.

Most estimates of storage life have been based on bulk fish, that is based on whole, or gutted, fish stored in ice or in chilled water systems or on fish frozen individually or in blocks or as fillet or mince blocks intended for further processing. The quality controller in the factory sets limits to the quality of the material entering the processing line and these limits will depend on the product being made and on the intended market. The starting material must be 'suitable for the process intended' in the PSL definition. This is the quality controller's or technologist's criterion for the limit of PSL and gives a storage life counted from the time of catching or harvesting or from freezing of the bulk material. The

'initially high quality product' in the IIR definition is the quality of freshly caught or harvested fish whether stored in ice or frozen immediately and stored.

Processing is not an end in itself and the technologist must bear in mind the interest of the ultimate consumer of the fish product. It must be at least 'suitable for consumption', a phrase which is similar to the public health inspectors' criterion of 'fit for consumption'. In the marketing context the phrase 'suitable' might be better replaced by 'acceptable'. In fact Jul in his recent book /10/, on p 53, equates 'acceptability time' with PSL and defines it as the 'maximum time during which a product would remain acceptable to the discerning consumer'. Though criteria of acceptability are probably nearest to the purpose for measuring storage lives they are not all that easy to translate into experimental protocols. The difficulties are in measuring acceptability and in selecting a panel to represent consumers, discerning or otherwise.

The bulk material may be processed into a prepacked consumer product and offered for sale in a store in a chilled or frozen cabinet. The producer is interested in how long the product can remain offered for sale, that is its shelf life. In this context the shelf life is the time since the, presumably, high quality product was packed until it becomes unacceptable to the consumer. It is the usual practice of processors when date stamping prepacked consumer products to count the allowable time from the time of packing which, in the case of frozen fish, can be far removed from the time of initial freezing of the bulk material.

The pressure of consumer interests have led to the introduction of open date stamping of packed foods in many countries. Some of the issues in date stamping have been discussed by Labuza /11/ though he refrains himself from defining shelf life in the book. The definition in the EC Directive, 'retains its specific properties' is different in principle from the others in that it relates to properties of the product itself and not to considerations of its use. It is a definition which the technologist could support since it satisfies the principle often expressed by them that the highest quality product is one that most resembles freshly caught fish.

4. RELATIONSHIP BETWEEN THE VARIOUS CRITERIA FOR DETERMINING THE END OF STORAGE LIFE

These various definitions and criteria if used with the appropriate experimental protocols, will almost certainly give rise to different estimates of storage life for a product under fixed storage conditions. I can illustrate the relationship between the various measures using as an example white fish stored in ice and results from work at Torry Research Station. The Torry scale /12/ for determining freshness by the flavour of a cooked sample is relevant and is listed in Table 1.

TABLE I

Freshness Scale of Flavour of Iced White Fish

10 'prerigor'; watery; slight sweetness	6 insipid; flavourless
9 sweet; meaty; creamy	5 slight sourness; slight musty, stale flavour
8 sweet, meaty, characteristic flavours but reduced in intensity	4 slight bitterness; sour; 'off'
7 weak neutral flavour	3 strong bitterness; sour; sulphide

The descriptions in this scale reflect the 2 modes of spoilage which are accepted as influencing the changes which occur during spoilage. In early phase of spoilage chemical reactions dominate changes in flavour, reducing the intensity of intrinsic flavours but not introducing any unpleasant flavours. Later the effects of bacterial activity produce spoilage flavours. There is a transitional period when the flavour is weak and insipid. The regression of flavour score on storage time is shown in Figure 1 with the regions of flavour change sketched in. The regression coefficient is -0.33 units a day /13/, that is a unit decrease in flavour over 3 days of storage.

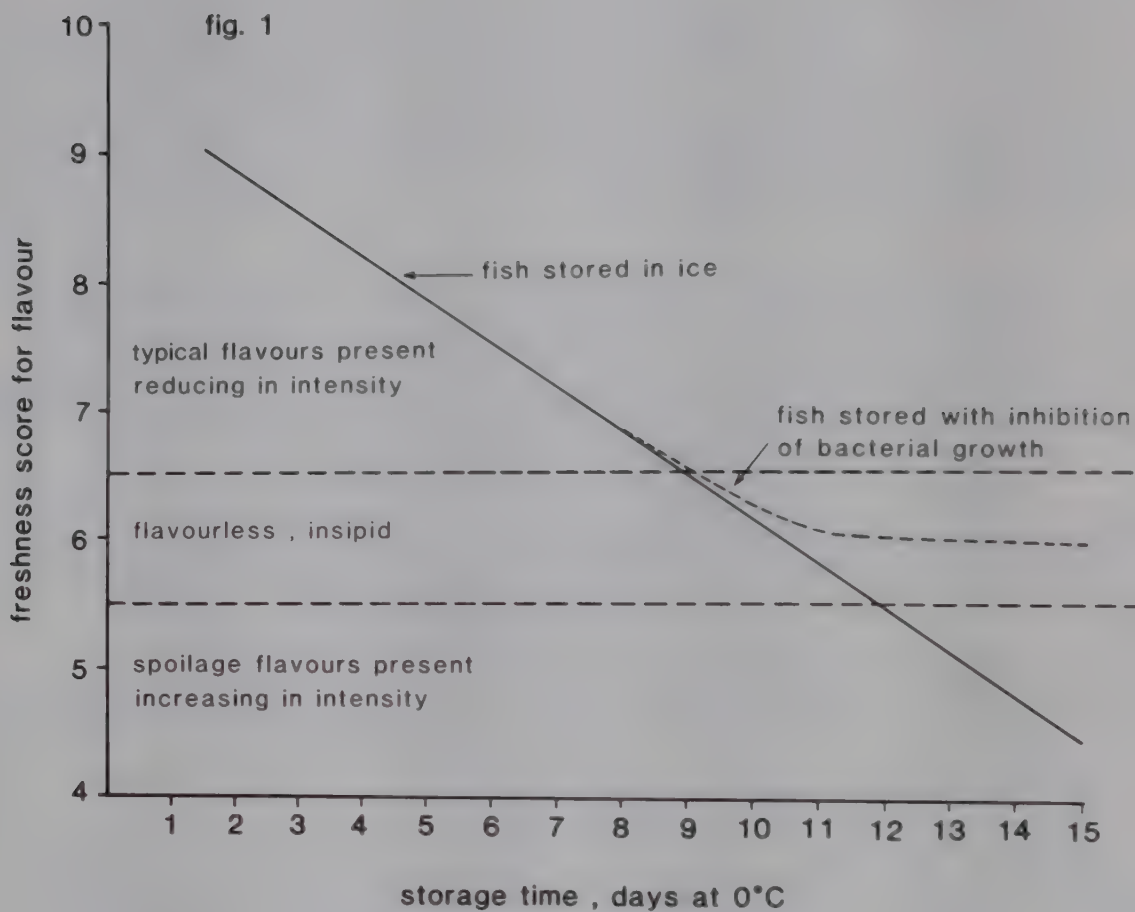


Fig. 1 - Effect of storage in ice on freshness flavour of cod

The Torry freshness scales do not directly rate the quality of iced fish but quality grades or limits can be defined on the scale. Baines and Shewan /12/ defined 'keeping time' as the period of storage in ice to a flavour score of 4.5, the point when they considered the fish became inedible. From Fig. 1 this criterion gives a storage life of 14½ days for gutted cod stored in ice.

Murray *et al.* /14/ when discussing quality of prepackaged fish products set the limit of acceptability at a flavour score of 5.5 but considered the limit of minimum freshness at the point of sale should be a score of 6.0. The difference allows for some spoilage between purchase and consumption. Working back from there they set the minimum freshness at the time of packing at score 7.0 and recommended that the shelf life, as defined earlier, of the packed product be 3 days.

Flavour scores of 7, 6 and 5.5 are, from Fig 1, equivalent to 7, 10 and 11½ days in ice. The 'suitable for process intended' criterion, if prepackaging is the process, results then in a storage life of 7 days. Once the pack is prepared its shelf life is 3 days. If the 'suitable for consumption' criterion is applied the storage life of iced cod is 11½ or 14½ days depending on an assessment of consumer acceptability. The EC Directive's criterion of retention of 'specific properties' would indicate a score 7 as the limit, perhaps pushing to score 6.

Changing the limit from score 7 to score 6 increases storage life by about 3 days for fish stored in ice but it can make a much larger difference in the case of some other forms of preservation. The rate of change of score down to about 7 is governed predominantly by chemical reactions. Bacterial growth has little effect and any process which inhibits bacterial growth does not alter rate of loss of freshness in this region. Such inhibition delays the time when spoilage flavours appear and reduces the rate of increase in intensity after that. Freshness scores with time follows the dashed line in Fig. 1 or show a break with a lower slope below score 7. This effect can be seen with irradiated fish or fish stored in high concentrations of CO₂. Fish handled under extremely hygienic conditions /15/ or sterile fish show the effect. If criteria for the end of shelf life are set at a score below 6 such treatments will considerably extend shelf life, setting it at 7 will not. This point may help to resolve some controversies over whether or not treatments involving bacterial inhibiting systems extend storage or shelf life.

These various estimates of storage life are derived by technologists based on an assessment of the changes that occur during storage and on their assessment of what consumers will accept; they are not derived from direct assessment of acceptability. An attempt has been made at Torry to measure acceptability directly using staff at the Station. Cod, haddock and whiting iced for up to 16 days were presented to a total of 105 people as fried fish fingers or as steamed fish in parsley sauce. Samples were rated on a 9 point hedonic scale /16/ where 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely. Differences in acceptability between species and between presentations were not large and the pooled results are shown in Fig. 2 as % of assessors showing some degree of liking. (Liking is defined as the proportion of ratings of 6 or greater plus half the number of ratings of 5.)

We do not find 100% liking by our assessors even with the freshest material. A % liking of around 85% for product of high quality is quite typical of the panel and has been observed over the 5 years or so that hedonic rating tests have been carried out here (see paper by Whittle *et al.* at this Conference). If it is considered that the 15% or so not liking the samples - even though they take part in the Torry tests - would not purchase or eat fish at all the consideration of the effects of storage should best be based on the remainder. Figure 2 shows a scale starting at 100% liking at 2 days in ice to facilitate reading of data rescaled in this way.

The proportion liking changes very little between 2 and 4 days of storage and decreases progressively after that. This trend should be comforting for fish technologists since it supports their stance that the best quality is that which most resembles freshly caught fish. The findings should also reassure the processor that improved quality in the form of fresher fish gives greater customer satisfaction. What this figure does not do is provide a criterion for deciding on the limit of storage life in ice. Close on 40% of assessors originally liking the fresh fish still liked fish stored for 16 days in ice so it would be unrealistic to set the limit at the time that all consumers found the product unacceptable.

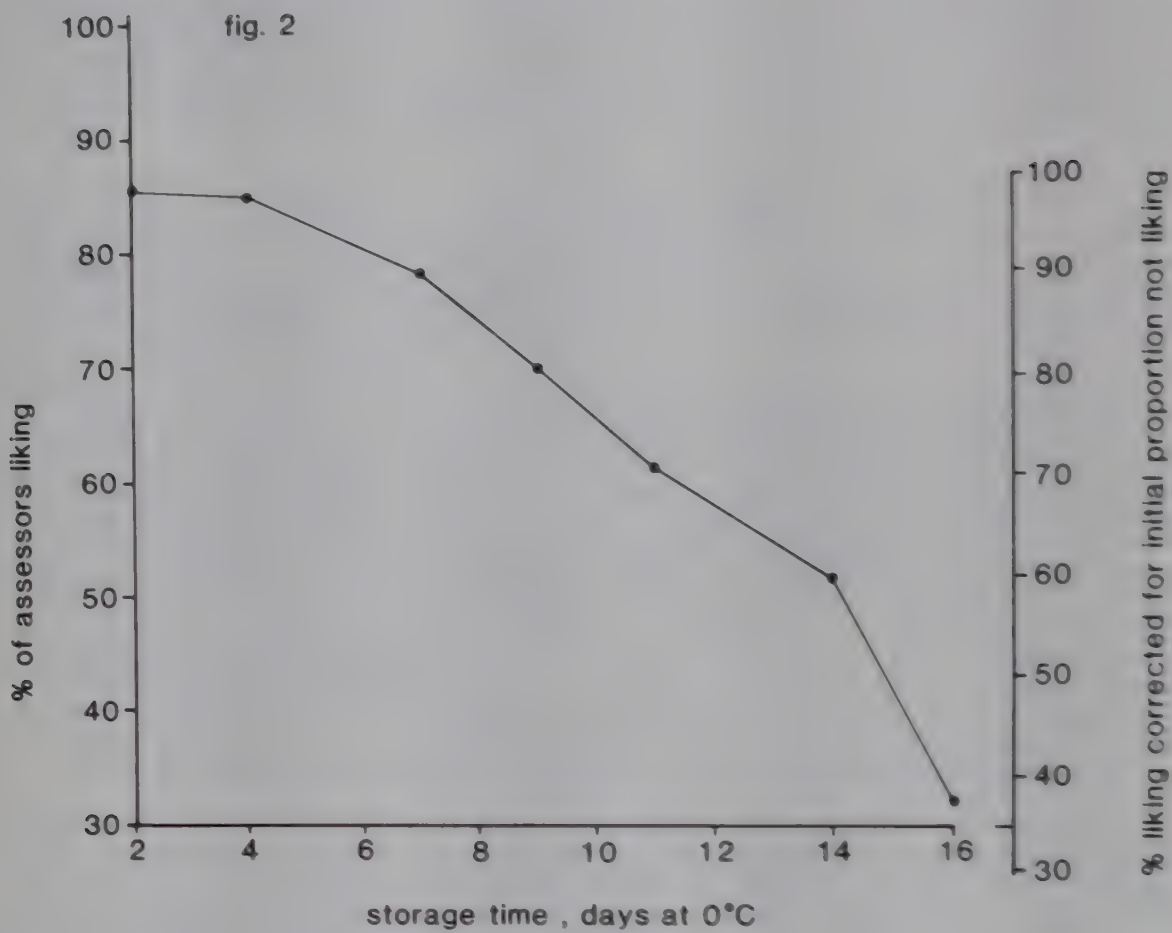


Fig. 2 - Effect of storage in ice on liking for white fish

A criterion of 'acceptable to the consumer' should therefore include some indication of the proportion of consumers which would be satisfied. It will also have to assume that the consumers are willing consumers of that product. Taking the response of the Torry population as an example the willing consumers are represented by the 85% expressing some degree of liking for very fresh fish. After storage for 10 days in ice, equivalent to score 6 on the Torry freshness scale, 75% of these consumers still like the fish. We do not know the responses of the Torry panel compared with the general population of consumers so we cannot say if this relationship holds outside of Torry but it does suggest that setting a score of 6 as the end of shelf life should result in products which are acceptable to a high proportion of consumers. Setting the limit at score 7, equivalent to 7 days in ice, increases the proportion liking to 91%.

It is much more difficult to set criteria for frozen fish. The organoleptic properties of frozen fish products change in intensity during storage and not in character. In white fish a characteristic cold storage flavour increases in intensity and in fatty fish rancid flavours increase in intensity. In all species texture alters during storage and is usually measured as an increase in toughness. Though technologists assume these changes are such that quality deteriorates and the product becomes less acceptable to the consumer there are no satisfactory criteria for deciding when the product has reached the end of its storage life.

The criterion in the EC Directive is difficult to apply since a frozen stored product does not really lose its characteristic properties; rather textural properties change but may remain within a characteristic range. Characteristic properties are overlaid and eventually masked by flavours produced during storage. If having characteristic flavour means the absence of off flavours then it has to be decided at what intensity level can these flavours be detected by the consumer. We are then back to the HQL definition.

Even if low intensities of off flavours or small changes in texture can be detected by the consumer it does not necessarily mean the product becomes less acceptable. The natural toughness of fish varies over quite a wide range and the consumer is probably tolerant of some increase of toughness during frozen storage as being within this natural variation. Kelly /17/ in fact has pointed out that unfrozen cod may not have the most acceptable texture and some degree of toughening during cold storage can improve acceptability. It is not clear to what extent cold storage flavours affect acceptability. Connell and Howgate /18/ concluded that in cold stored fish flavour had more influence on acceptability than texture and Kelly /17/ also has pointed to the importance of flavour but their conclusions are based on results from laboratory panels. The findings of Mackie *et al.* /19/ show that a large panel of assessors from outside of research laboratories do not seem to be influenced by changes in cod products stored at -18°C for up to 1 year.

Many investigators have set limits to the storage lives of frozen fish products on the basis of storage trials in which quality has been measured by sensory methods. A study of the literature shows that the typical procedure is to employ a small panel consisting of a small number of expert assessors rating the products on a quality scale. These scales have a point described in various ways but 'unacceptable quality', 'fair', 'borderline', are quite typical and this point is taken as the criterion of the end of shelf life. It must be pointed out that this criterion is defined by the panel itself and is not a measure of acceptability outside of that group.

5. CONCLUSIONS

The main conclusion that can be drawn from this brief review is that storage life needs to be better defined; the present definitions leaves much to be interpreted by the user. In spite of the considerable effort that has gone into measuring storage lives of fish in both the wet and in the frozen states there is no consensus of the criteria for deciding on the limit of storage life. It is also the case that the quality of stored products has been assessed by small panels of expert assessors in technical institutes who themselves define the criteria for the end of storage life.

Technologists are not ignorant of the response of consumers to the quality of fish but it has to be accepted that extrapolations from expert assessment to consumer acceptance have not been tested in any quantitative way.

There seems to be a move to save storage life defined to give 2 levels of quality. The level is directed towards the discerning consumer in which the consumer has legislation governing shelf life stipulating the other is at the level of which is acceptable for consumption and is at a lower quality than the former. These 2 storage times for a product have been expressed mostly with regard to wet fish and it arises from a scan of the literature on storage life of whole stored fish that most investigators have used the lower limit. I feel it would not be difficult to get a consensus among fish technologists on definitions for these 2 levels.

It is particularly difficult to establish objectively criteria for defining the limit of storage life for frozen fish. Sensory and non-sensory methods for measuring storage life of frozen fish products are not readily as well established as they are for still stored fish and there does not appear to be a clear relationship between the properties measured on these basis and consumer acceptability. There is clearly a need for more research on sensory, and particularly life sensory methods, for measuring the quality of frozen fish and also determining the quality factors that influence consumer acceptability.

REFERENCES

1. C.A.M. LIMA DOS SANTOS: The storage of tropical fish in ice - a review. *Tropical Science*, 23, (1962), 97-127.
2. J.B. LANE: Time-temperature tolerance of frozen seafoods. 1. Review of some recent literature on the storage life of frozen fishery products. *Food Technol.*, 18, (1964), 1100-1106.
3. INTERNATIONAL INSTITUTE OF REFRIGERATION: Recommendations for the storage of perishable produce. IIR, Paris, (1979).
4. INTERNATIONAL INSTITUTE OF REFRIGERATION: Recommendations for the processing and handling of frozen foods. 2nd edition. IIR, Paris, (1971).
5. COUNCIL OF THE EUROPEAN COMMUNITIES: Council Directive of 18 December 1975 in the approximation of the laws of Member States relating to the labelling, presentation and advertising of foodstuffs for sale to the ultimate consumer. (79/112/EEC). *Official Journal of the European Communities* No. 132, (1975), 1-15.
6. UNITED KINGDOM: STATUTORY INSTRUMENTS OF 1964 (1975). The Food Labelling Regulations 1964. H.M.S.O., London, (1975).
7. E.J. MCLELLAN: Quality and stability in frozen fruits and vegetables. In "Quality and stability of frozen foods", A. S. Tamplin, A. M. Taylor and E. J. Mclellan, eds. Wiley Interscience, New York, (1966), 85-116.
8. E.J. MCLELLAN: Can shelf life be measured? *Food Technology in Australia* 14, (1962), 149-152.
9. E. MOWAT: Measuring the storage lives of animal and frozen fish products. *Proceedings of the 11th Congress of the IIR, Paris, 1961*. (To be published).
10. E. MOWAT: The quality of frozen foods. *Scientific Press, London and New York*, (1962).
11. F.F. LAMMIE: Shelf-life dating of foods. *Food and Nutrition Press, Inc., Westport*, (1962).
12. J.B. LANE and F.I.W. SWAIN: Sensory methods for evaluating the quality of white fish. *Laboratory Practice*, 14, (1965), 190-194.
13. J.B. LANE, D.W. GILSON, A.J. LAMSON and E.J. MCLELLAN: Comparison of methods of freshness assessment of wet fish. 1. Sensory assessment of frozen experimental fish. *J. Food Technol.*, 11, (1976), 75-88.
14. E.J. MCLELLAN, D.W. GILSON and F.I.W. SWAIN: Quality control aspects of packaged fish and frozen fish. In "Quality assessment and quality control", A. Freaser, ed., Fishing News Books Ltd., Surrey, England, (1971), 20-30.
15. E.J. MCLELLAN, D. GILSON, L. GILSON, A. LAMSON, A. PETERSON and F. GILSON: The influence of hygiene in catch handling on the storage life of cod and plaice. *J. Food Technology*, 9, 1978, 10-21.
16. E.R. PERYAN and F.J. PILGRIM: Hedonic scale method of measuring food preferences. *Food Technol.*, 10 (1956), supplement, 1-14.
17. I.B. KILBY: Quality in frozen cod and limiting factors on its shelf life. *J. Food Technol.*, 4, (1969), 95-103.
18. J.V. POWELL and F. MOWAT: Consumer evaluation of fresh and frozen fish. In "Fish Inspection and Quality Control", A. Freaser, ed., Fishing News Books Ltd., Surrey, England, (1971), 155-159.
19. I.W. WALKIE, P. HOWGATE, A. CRAIG, W.W. LAIRD and A.H. RITCHIE: Acceptability of frozen stored consumer fish products. *Food Technology*, 1968, 100.

A PROPOS DE LA DEFINITION ET DE LA MESURE DE LA DUREE DE CONSERVATION DU POISSON REFRIGERE ET DU POISSON CONGELE : BREVE REVUE

RESUME : Il a été publié de très nombreux articles dans les revues techniques sur la mesure de la durée de conservation du poisson frais et du poisson congelé, mais les chercheurs fournissent rarement la définition de ce qu'ils entendent par durée de conservation. La durée de conservation a été estimée de nombreuses façons suivant le contexte dans lequel cette expression est utilisée. Les limites de la durée de conservation du poisson glacé peuvent être établies d'après les modifications de la saveur au cours de la détérioration mais il n'est pas possible de définir les limites d'entreposage du poisson congelé.

DISCUSSION

C.R. JOHNSON (U.S.A.) - We experienced problems with Marketing Technologists not understanding and not able to use sensory evaluation, hedonic scores or storage life data developed by Food Technologists. Would it, therefore, be reasonable to assess whether consumer (100 to 400 samples) evaluation of acceptable or non-acceptable would give better marketing information ?

P. HOWGATE - Hedonic rating tests attempt to measure subjective response - the subject's liking for a product. This liking will vary widely among subjects in a population - typically, we find in our trials a range of scores from 1 to 5 for a product rated on the Keryan - Pliginsk 9-point hedonic scale. A large panel of the size you mention should be used, although the hedonic scale is often used as assessing acceptability because of its directness and simplicity. I believe it is deceptively simple. The response given by the subject is not absolute but is dependent on the context of the assessment. This context not only includes the physical situation but also things like the way the products are presented, the instructions given to the subject and, importantly, the expectations of the assessor. A product is assessed relative to other products presented at the same time or the memory of similar products and there is also an element of anticipating the results of the experiment and even of pleasing the experimenter.

TIME TEMPERATURE MONITORING & QUALITY INSPECTION
FOR A QUICK FROZEN FOOD MANUFACTURER.
RECENT CHANGES IN DESIGN & PRACTICAL CONSIDERATIONS FOR FISH PRODUCTS

M. SANDERSON-WALKER
Birds Eye Wall's Ltd. (United Kingdom)

1. THE UK MARKET

The retail sales of quick frozen foods in the United Kingdom continue to increase, and it is estimated that the Quick Frozen Fish tonnage has increased by 27% between 1980/1984. The traditional distribution system from a large number of small depots to the retail store is being replaced by Regional Distribution Centres; (with a few satellite depots). These stores may be part of the manufacturers, retailers, or a third party general distribution system. The retail outlet pattern continues to evolve with the large superstores continuing to develop together with a small number of "convenience stores" and home freezer deliveries. There have been mergers and changes in ownership of a number of retail chains and wholesalers, with several new cold stores having been opened. (See Fig. 1). /1/.

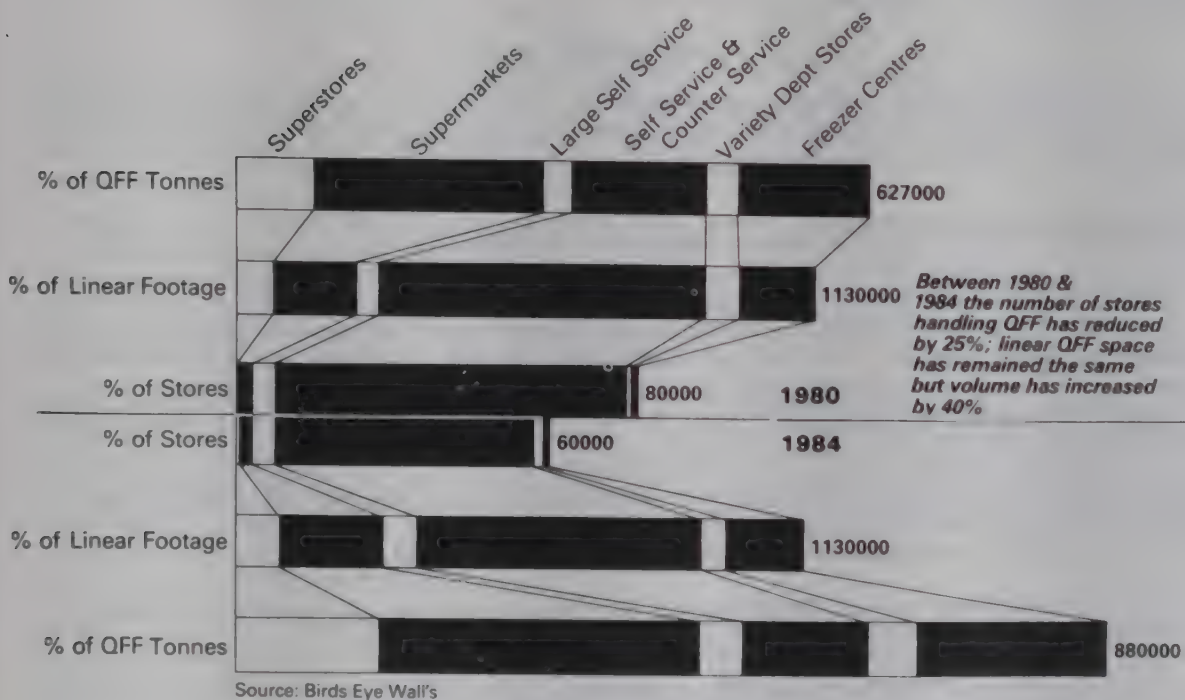


Fig. 1 Volume of Frozen Food Sales by Outlet Type 1980 and 1984

The popularity of "added value products" especially Fish in Sauce, Fish in Batter, and Fish with Pastry/Pasta, have increased the branded manufacturers concern to enhance product quality through:

- Better temperature control
- Improved design and performance of packaging
- Improved stock rotation.

This paper looks at some of the quality improvements which have been taking place and considers some of the problems which need resolution.

2. PRACTICAL STORAGE LIFE

In 1984 in his book entitled the "Quality of Frozen Foods" Jul /2/ stated that "estimating the keeping times for fish is particularly difficult because differences exist between fish for the same species from different areas. Besides, fish is rarely frozen immediately after capture". A manufacturer has to have regard to many factors in order that a consumer receives a good quality product. The effect of time and temperature on quality are increasingly well known although estimated Practical Storage Lives at the same temperature by different researchers vary greatly. The writer agrees with Jul that the estimation of Practical Storage Life must be carried out with products in the packaging which will be used for actual distribution. Manufacturers control the product, its processing and packaging but have little or no control over the temperatures or time at the retail level, and in household appliances. Because of the differences in findings at different laboratories, and the variation in retail storage conditions, it is important that a branded manufacturer estimates storage lives adequately. Accelerated storage life testing is a useful method for predicting PSL particularly when a marketing launch is required soon after the development has been completed /2/.

Improvements in those factors under the producer's control continue to be made often as a series of small steps. It is sometimes easier to make significant improvements when equipment is being replaced as this allows the "quality of design" to be changed radically. Some of these changes take several years to complete, as in the case of a vehicle fleet, when a number of units become fully depreciated and are replaced each year.

3. BETTER TEMPERATURE CONTROL

For most fish products the principle that the colder the storage temperature the longer the Practical Storage Life applies. Innovations in transport and cold storage are leading to improved product temperature control. These include:

Regional Distribution Centres. A cold store with up to 1,000,000 cubic feet (approx 28,000M³) replacing several smaller depots. Pallets are located in narrow aisle single entry racking with good air circulation around them. Blown air is generally at -26°C/-29°C, some 3°C colder than the depots which have been replaced. The stores have enclosed loading banks with port doors, and the newest have a pallet conveyor accepting stock. The latter eliminates the use of trucks to transfer from trunker to cold store through large open doors and reduces the entry of warm moist air into the store. (Where possible retained small depots have had their air temperatures lowered to -26°C).

Trunkers. Vehicle design in the U.K. is evolving with the increase in total allowed weight. Drikold has been used by some companies for many years, and continues to provide a simple and effective refrigerant for many types of journey.

Pressures to provide colder temperatures of delivery define the need for a trunker which can:-

- Carry up to 20 and possibly 22 pallets.

- Operate from a diesel electric refrigeration system when mobile.

- Run from a three phase electrical socket when parked at a company's site.

- Give a temperature holdover of many hours if the plant is not operational.

Provide good product temperature performance due to demands from third party cold stores for colder delivery temperatures.
Recover the air temperature quickly after door openings as multi-drops are required.
Obey the noise regulations as defined by the local planning authority.

These objectives are being achieved by the use of trailers which are fitted with tubes carrying a eutectic solution which can be cooled by a refrigeration system at the front bulkhead to -30°C . An auxiliary fan circulates air over an evaporator and is used to aid air temperature recovery when necessary. The power is obtained either from a diesel generator mounted near the rear wheels, or via a three phase electrical supply lead. Defrosting of the coils is required occasionally to remove snow and ice. Results recorded to date indicate a good ability to deliver products colder than -18°C . and operate a "tramping" service throughout the U.K delivering products and collecting Raw Materials for delivery to a factory.

Retail Delivery Vehicles. The latest designs are intended to carry pallets, roll pallets, or individual outers packed directly within the vans. The eutectic system is also used in these vehicles and is "charged" by being plugged into a three phase electrical socket when at a company's site. The good performance of these vehicles requires the use of strip curtains in the door space to minimise the entry of warm moist air and periodical defrosting of the whole system is necessary.

Retail Display Cabinets. British Standard 3053 for retail display cabinets has been updated and was re-issued in 1983 bringing it into line with the International Standard No. 015 5160. The temperature requirements remain the same and it is noticeable that sampling by the Humber Laboratory from retail cabinets gave about 18% of packs tested warmer than -12°C /3/ which is the warmest temperature allowed in the standard under test conditions.

Home Storage. In the U.K. the concept of "star marking" is well established both on household equipment and packets. Manufacturers include star markings storage instructions when considering the practical storage life of their products. It has been estimated that 69% of households in the UK owned a home freezer in 1984 and consumed 84% of Quick Frozen Foods /1/.

4. PACKAGING FOR FINISHED PRODUCTS

The protection of an intrinsically high quality product is an extremely difficult design problem, and is becoming more complicated with increasing mechanisation and faster speeds of production. Examples of factors which must be considered are:

Sales Order. This can range from as little as 6 packets to a complete trunker of products. To achieve control of such variations in order size my Company shrinkwraps products in units of 6 or 12 packets and, increasingly automatically bundles 4 units together with stretchwrapping material to produce a "case" of 24 or 48 packets. These will be assembled onto 1,200 x 1,000mm pallets to give the maximum occupancy within the permitted height of 1676mm. The whole pallet is then spiralwrapped prior to storage.

Fragile Irregular Products. The large scale production of products such as Fish in Batter is inevitably accompanied by some variation in shape albeit within the process specification tolerances. Cartons containing such products must be designed to have a slight head space, but yet be strong enough to withstand the rigours of the distribution chain.

Boil in Bag Products. Fish in Sauce products have become increasingly popular either as single portion sachets in a carton or as multi packs. Such materials have to be able to withstand freezing tunnels at -35°C, fluctuating temperatures especially in retail handling and +100°C when placed in boiling water to prepare the product.

The Distribution Factor. With no outer cases (fiberites) the packets and products have to be self supporting on the pallets. Transit tests are needed for each new or changed design to ensure acceptability. Each step in the packaging operation must be carried out correctly to avoid failure.

5 IMPROVED STOCK ROTATION

A manufacturer usually produces several products in different pack sizes on the same production line, on different days. An example is that Fish in Sauce could be made from cod, haddock or plaice for more than one brand with sauces containing butter, parsley or cream. The pack size may be 1 sachet per carton or 4 or more sachets per carton.

The production requirements vary from week to week, and some packs sell more quickly than others. Variable raw material availability, public holidays, marketing promotions, and line overhauls, influence a minimum level requirement for stock. Strategic stock planning is becoming a science and the use of computerised re-ordering systems using exponential smoothing factors are a great help. These are designed to allow packs to pass quickly through each stockholding point. Sensitive fish products need to be monitored for age whilst under the manufacturers control. To satisfy a national distribution system plus exports, a notional stockcover of about 42 days is necessary with up to 10 days cover being in each Regional Distribution Centre.

The survey carried out by The Humber Laboratory /3/ gave figures for my own Company's Branded Cod in Sauce pack with a log mean age of 28 days at the factory, 37 days at the depots and 77 days from shop samples.

Reductions in the number of stockholding points plus the introduction of stock location computers will be of assistance in improving stock control and rotation still further. There is a large commercial incentive to achieve these objectives to allow reductions in working capital together with a saving in cold store space, operating costs, and interest on capital employed.

The U.K. Association of Frozen Food Producers endorsed a code of practice /4/ whereby outers are marked with a rotation code 25mm in height. This consists of a month and year symbol. Thus, the symbol 1/85 indicates that stock was produced in January 85. It is intended as a aid to our own staff, third party cold store operators, and most of all to retailers. It is hoped that non-members of the UKAFFP will also introduce this symbol as their multiplicity of case coding systems do not allow good stock rotation in cold stores.

The introduction of a Quality Code panel on retail packets has greatly helped product identification from a management viewpoint by indicating both the computer stock reference, the factory, and coded date of production; as well as special information required by the factory, eg. production period or filling machine. (See Fig. 2).

In case of dissatisfaction please return our quality code panel to the address below.

Birds Eye Wall's Ltd.,
Walton-on-Thames, Surrey, England
Produce of the United Kingdom.

DW

15A

Fig. 2 Quality Code Panel on Packets

6. RETAIL STORAGE/QUALITY

In 1979 the writer /5/ made a plea for better stock rotation in retail display cabinets using the "first in first out" system. A recent analysis of a sample of fish condition complaints from consumers has shown that 8% were between 9 and 12 months old, and only 3% more than 12 months old. The reduction in condition complaints due to the failure of control of time and temperatures as reported in 1979 has not continued, and the trend is increasing following the very hot summers in the United Kingdom in 1983 and 1984. A sympathetic and rapid response to customer complaints of "sour" or unusual fish flavours can redress the grievance. However, U.K. consumers are increasingly demanding only first class quality, and blame the producer as it is his name on the carton. Therefore it becomes a matter of serious concern to make certain that turnover in the retail stores is as rapid as possible.

To advise retailers, guidelines for handling quick frozen foods have been issued as an illustrated card in January 1984 to all accounts receiving our price list. /6/. Regrettably these have not been reported as having any great effect upon the level of consumer complaints, and it has been felt necessary to obtain better information on the rotation of stocks within retail stores.

To this end several deliveries of Fish Finger cartons (and some other products) were specially coded, and with the agreements of a small number of stockists, their rate of sale was checked weekly in the cabinets. The data was supplied to Stirling University following preliminary analysis. A computer systems model of the retail operation has now been developed to provide guidance for future quality planning. This is described by Earl et al /7/ at this conference.

There is a great need for better handling, control of temperatures, and improved stock rotation at this stage. If this could be achieved then it should be possible to increase sales further, and allow the development of products which cannot easily be marketed today because of the need to be able to withstand the loss of quality in either the back up store or the cabinet.

7. CONCLUSION

This paper has considered changes which have occurred in the quality assurance of quick frozen fish products distribution in the U.K. in recent years. Whilst manufacturers have continued to improve the quality and reliability of the operations under their control, the consumer is responding by demanding even higher standards. Changing consumer lifestyles are increasing this trend through /1/.

The growth in the number of working women
Increased leisure activities
Decline in traditional family meal occasions
Increased exposure to and demand for a wider variety of foods plus
A desire for convenient, balanced, easy to prepare meals.

Retail handling is a key factor in the maintenance of product quality, and a constructive dialogue has been taking place between manufacturers and retailers organisations in view of the draft EEC Directive for quick frozen foods.

REFERENCES

1. Birds Eye Wall's Ltd. - Report Building the Competitive Edge - 1985.
2. Jul, M The Quality of Frozen Foods - Academic Press 1984 ISBN 0-12-391980-0
3. Humber Laboratory 1983-4. Private Communications.
4. UK Association of Frozen Food Producers, 1 Green St., London, Code of Practice for Rotation Coding.
5. Sanderson-Walker, M. (1979b), Int. J. Refrig., 2(2), 97-101.
6. Birds Eye Wall's Ltd., - Handling Code Card for Retailers 1984.
7. Earl, Allen and Kindleysides - Simulation Modelling of Frozen Food Distribution - Retail Operations and their effect on quality. This meeting, page 000.

SURVEILLANCE AUTOMATIQUE DE LA DUREE ET DE LA TEMPERATURE ET INSPECTION DE LA QUALITE POUR UN FABRICANT D'ALIMENTS SURGELES. MODIFICATIONS RECENTES DE LA CONCEPTION ET CONSIDERATIONS PRATIQUES POUR LES PRODUITS A BASE DE POISSON.

RESUME : La distribution de détail des surgelés au Royaume-Uni poursuit son évolution vers un accroissement des grands centres de distribution. Le besoin de répondre aux commandes de magasins de tailles diverses a favorisé le développement de nouveaux centres de distribution régionaux opérant à des températures plus basses; des améliorations de la conception des camions interurbains et des engins de livraison de détail assurant également une température plus basse des produits. Le conditionnement prend une importance croissante pour protéger les produits, ainsi que pour fournir un récipient dans lequel les nouveaux produits à base de poisson peuvent être cuits. Une meilleure rotation des stocks devient possible grâce à des systèmes informatiques pour localiser les palettes et les emballages extérieurs à condition qu'ils portent la codification appropriée. Des "Codes de Rotation" pour les emballages extérieurs et un label de qualité pour les paquets ont été introduits pour aider à la gestion des stocks, au repérage des produits et à leur identification. Dans le secteur de détail, il faut améliorer le contrôle de la température, ainsi que la manutention et la rotation des stocks. Une carte de manutention codée pour le détaillant a été émise et un appui a été accordé à un projet de recherche pour un système informatique de vente.

DISCUSSION

S.J. JAMES (U.K.) - It has been previously suggested that temperature fluctuations are acceptable. Do you think that fluctuations in the existing cold chain affect the quality of your products ?

M. SANDERSON WALKER - Work by, Hawkins, Pearson and Raynor published in 1973 shows the effects of temperature fluctuation on quality. This showed that fluctuations at a lower temperature were less harmful than fluctuations at higher temperatures. The method of determining quality changes was subjective, with a scoring scheme used to show significant differences between treatments.

SECTION II

EVOLUTION CHIMIQUE AU COURS DE
L'ENTREPOSAGE DE PRODUITS CONGELÉS

CHEMICAL CHANGES IN FROZEN STORAGE

EFFECT OF THE TEMPERATURE FLUCTUATION ON THE MEAT QUALITY OF FROZEN MACKEREL (Scomber tapeinocephalus)

SHANN-TZONG JIANG, CHING-YU TSAO AND MING-LANG HO
Department of Marine Food Science, National Taiwan College of
Marine Science & Technology, Keelung, Taiwan

INTRODUCTION

The ideal temperature condition accepted by most people is to keep food at a constant -18°C or lower temperature. It is usually possible to maintain the -18°C or lower temperature but impossible to keep the temperature constant. Sometime during the processes of handling, transporting, and storage, the temperature of frozen foods frequently changes. Depending upon the situations, the foods may undergo several and even a large number of fluctuation in storage temperature with various magnitudes before it reaches the ultimate consumer.

No distinct deterioration of palatability in some vegetables and fruits stored for 6 months at a temperature fluctuating from -18°C to -28.8°C was observed, as compared to a constant storage temperature of about -20.5°C /1/. The study by Hustruid et al. /2/ indicated that temperature fluctuations below -18°C were not important factor causing the deterioration of color, texture, and flavor of snap beans, strawberries, ground beef and pork. However, desiccation of meat wrapped in waxed locker paper was much greater under fluctuating storage condition than at constant -18°C . The palatability and volatile acid numbers (for showing relative freshness) of mackerel fillets stored at fluctuating temperatures of -17.7°C to -23.3°C fell below these of fillets held at constant -17.7°C and -23.3°C /3/. The quality of cod, horse mackerel was affected by being stored at fluctuating temperature above -10°C /4,5,6/. However, no distinct changes in quality of frozen boxed beef was found from both constant -23°C and di-thermal storage at -23°C (diurnal) and 21°C (nocturnal), 21°C (diurnal) and -18°C (nocturnal) /7/.

Fluctuation in storage temperature will originate the recrystallization and accretion of ice crystal during storage and transport of frozen foods /8,9,10,11,12,13/. They involve the growth of the smaller ones which tend to disappear, with the consequent reduction of the total number of crystals and enlargement of average crystal size. The quality of products of which have been correctly frozen is then, deteriorated. The recrystallization has been demonstrated in a variety of foods /14,15,16,17,18,19,20/. The rate of recrystallization in beef liver tissue during frozen storage had also been determined by Sy and Fennema /21/.

Bramsnaes /12/ indicated that fluctuations in temperature per se have little effect on the quality, except for products with unstable texture and in-package ice formation. Fish muscle proteins was considered to be very unstable, especially during frozen storage /8,9,10,11,22,23,24/. The gel-forming ability of kamaboko (a kind of minced fish products) is mainly affected by the degree of protein denaturation during frozen storage and transport /8,9,10,22,24,25,26/. Recently, it has been reported that mackerel, amberfish, and sardine is possible to be as raw materials for producing minced fish products /27,28/. This study was monitored to investigate the effect of temperature fluctuations on the meat quality and kamaboko gel-forming ability of mackerel during frozen storage.

MATERIAL AND METHODS

Fresh mackerel (Scomber tapeinocephalus) stored with ice for 18-24 hours after being caught was eviscerated and deboned. After being washed, samples were frozen to a body temperature below -18°C using an

air blast freezer (temperature: -35°C , wind velocity: 3.0 m/sec). The frozen samples were packaged with polyethylene bags and divided into 5 groups. They were stored at -30°C , temperature fluctuating between -30 and -20°C at 3 and 7 day intervals, and between -30 and -10°C at 3 and 7 day intervals, respectively. After 2, 6 and 10 weeks storage, samples were removed and thawed in running water (20°C) until the body temperature reached 0°C . They were subjected to the following measurements.

Quality Assay:

Extractability of 0.6 M KCl-soluble proteins:

To 10 grams of fish meat, 180 ml of chilled 0.6 M KCl solution ($\text{pH}:7.2$) was added. It was homogenized using a Waring Blender subjoined with a baffle plate for 2 minutes. Centrifugation was performed under 5000 X G, 0°C for 30 minutes. The supernatant was made to 300 ml with 0.6 M KCl solution, and used as 0.6 M KCl-soluble proteins solution. The concentration of protein was determined by Biuret method modified by Umemoto /29/. Extractability of the 0.6 M KCl-soluble proteins was expressed as milligrams of protein per gram of muscle.

Extractability of actomyosin:

The actomyosin was extracted according to Noguchi /25/. The concentration of actomyosin was determined using Biuret method modified by Umemoto /29/. Extractability of the actomyosin was expressed as milligrams of actomyosin per gram of muscle.

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis analyses:

In order to investigate the conformational change of the muscle proteins during frozen storage, the SDS-polyacrylamide electrophoresis was performed according to Weber and Osborn /31/. The 0.6 M KCl-soluble proteins was incubated at 40°C for 2 hours in a solubilizing solution consisting of 0.01 M sodium phosphate buffer ($\text{pH}:7.2$), 1 % SDS, 25 % glycerin and 2 % mercaptoethanol. The solubilized salt-soluble proteins was then, dialyzed overnight at room temperature against 0.01 M sodium phosphate buffer containing 0.1 % SDS, $\text{pH } 7.2$. On the top of polyacrylamide gel, 0.02 ml of 0.05 % bromo-phenol-blue and 0.03 ml dialyzed protein solution were dropped with micropipet.

The polyacrylamide gel was prepared basically according to Weber and Osborn /31/. The concentration of polyacrylamide was 10 %. After electrophoretic run in 0.1 % SDS-0.1 M sodium phosphate buffer (8 mA each gel), the gels were stained overnight with 0.12 % Coomassie blue-50 % methanol-9.2 % acetic acid solution. They were then, destained with 50 % methanol-7.5 % acetic acid solution for 8-10 hours, as recommended by Seki /32/.

Distance scanning at the wavelength of 585 nm, which the staining solution has maximum absorbance, was employed to analyze the size and position of bands on the gel by using an UV-VIS Micro-processor-Controlled Spectrophotometer System 2600 (Gilford Instrument Lab. Inc.). The areas of peaks were automatically computed and used as the band size for calculating the percentage of each protein fraction.

Physical assessment:

The kamaboko was prepared from these freeze-thawed fish. After being skinned and deboned, samples were passed through a pre-chilled chopper. These chopped meats were then, washed with 5 volumes of tap water for 4 times and 5-6 minutes each time. 0.1 % Na_2CO_3 and 0.3 % NaCl solutions were used in the first and last washing treatments. Draining was done by employing a centrifuger. The moisture content was adjusted to 80 % by adjusting the speed of centrifugation. These chopped meats were strained by pressing through a strainer to remove the debris and scale. They were ground for 10 minutes and then 2.5 % NaCl, 0.2 % polyphosphate 2DK (mixture of 50 % Na-polyphosphate and 50% Na-pyrophosphate) and 3 % of corn starch were added and continued to grind for 20 minutes. These ground meat sols were stuffed into polyvinylidene chloride casings and sealed. Thermal treatment was done in 90°C water for 30 minutes. After being cooled sufficiently, samples were stored at 5°C overnight, and then subjected to the kamaboko quality assay.

The quality of kamaboko gel-forming ability was evaluated by measuring the gel-strength and folding test. The gel-strength was determined using San Kagaku R-UDJ type Rheometer (diameter of the plunger was 5 mm) and expressed as : gel-strength (g.cm) = breaking force (g) X deformation (cm). Folding test was also done according to Okada et al. /30/, and graded as: AA : No cracks occurred when folded into quadrants; A : No cracks occurred when folded into semi-circles; B : Cracks occurred when folded into semi-circles; C : broken into two pieces when folded into semi-circles.

RESULTS AND DISCUSSION

Effect of the temperature fluctuation on extractability of 0.6 M KCl-soluble proteins and actomyosin:

TABLE I

Effect of the temperature fluctuation on extractability of 0.6 M KCl-soluble proteins and actomyosin (mg/g of meat) of mackerel during frozen storage

sample	fresh		storage time (wk)					
	SSP*	AM*	2	AM	6	AM	10	AM
A**	180a***	85a	170a	82a	157a	77a	142a	65a
B	180a	85a	160ab	79a	144b	74ab	116c	48b
C	180a	85a	165ab	78a	146b	72ab	124b	52b
D	180a	85a	154b	75a	124c	64c	86e	40c
E	180a	85a	158ab	74a	133bc	69bc	100d	48b

* SSP: salt soluble-proteins; AM: actomyosin

** A: stored at -30°C; B: stored at a temperature fluctuating between -30 and -20°C at 3 day intervals; C: stored at a temperature fluctuating between -30 and -20°C at 7 day intervals; D: stored at a temperature fluctuating between -30 and -10°C at 3 day intervals; E: stored at a temperature fluctuating between -30 and -10°C at 7 day intervals.

*** Mean values in the same column bearing unlike letters differ significantly (P<0.01).

As shown in Table I, the 0.6 M KCl extractable proteins and actomyosin were 180 and 85 mg/g in fresh mackerel, respectively. The extractability of 0.6 M KCl-soluble proteins of samples stored at constant -30°C decreased gradually during storage. That of samples stored at temperature fluctuated between between -30°C and -20°C, -30°C and -10°C significantly decreased after 6 weeks storage as comparing with that of control (stored at -30°C). The rate of decrease of 0.6 M KCl-soluble proteins in samples stored at temperature fluctuating between -30 and -10°C was faster than that of fluctuating between -30 and -20°C, in spite of their fluctuating frequency. As depicted in Table I, the extractability of 0.6 M KCl-soluble proteins in samples stored at temperature fluctuated between -30 and -10°C at 3 and 7 day intervals was significantly different from those stored at constant -30 °C and fluctuating between -30 and -20°C after 6 weeks storage. However, that of samples stored at temperature fluctuated between -30 °C and -20 °C differed significantly from those stored at constant -30 °C after 10 weeks storage.

As shown in Table I. The extractability of actomysin of samples stored at temperature fluctuating between -30 and -10 °C was significantly different from that of samples stored at constant -30°C and fluctuating between -30 and -20 °C after 6 weeks storage, while that of samples stored at temperature fluctuated between -30°C and -20 °C differed significantly from that stored at constant -30 °C after 10 weeks storage (p < 0.01).

As indicated in studies by Kozima et al. /5/ and Nakamura /6/, the quality of cod and horse mackerel was affected by being stored at fluctuating temperature above -10°C based on measuring the changes in salt-soluble proteins nitrogen, volatile base nitrogen and K-value

(an index for evaluating the freshness). Nakamura /6/ point out that the quality of halibut and horse mackerel deteriorated with the increase of fluctuating frequency and amplitude during frozen storage. In this study, extractability of salt-soluble proteins and actomyosin decreased with the increase of temperature fluctuating amplitude and frequency. The extractability of 0.6 M KCl-soluble proteins and actomyosin of samples stored in a temperature fluctuated at 7 day intervals was higher than that of 3 days intervals.

Effect of the temperature fluctuation on the electrophoretic diagram of 0.6 M KCl soluble proteins:

The changes in the electrophoretic separation of 0.6 M KCl-soluble protein of these samples during frozen storage were expressed schematically in Figure 1. The percentage of the myosin heavy chain and actin (as quantity of actomyosin) was calculated by dividing the areas of myosin heavy chain and actin to the total areas. As shown in Figure 1-A, the percentage of actomyosin in salt-soluble proteins was 46.20% in fresh state, and decreased gradually to 33.40% (Figure 1-B) during 10 weeks storage at constant -30°C .

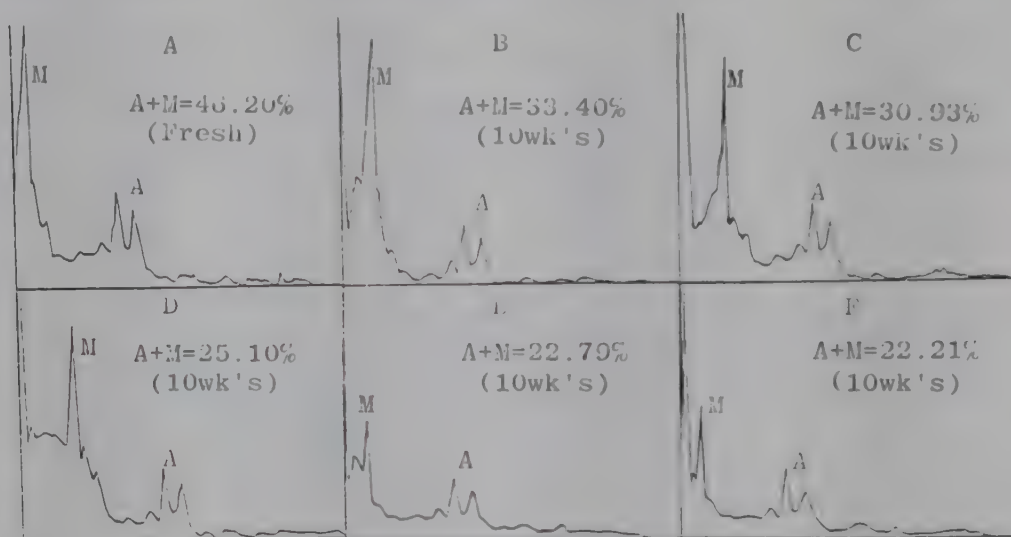


Figure 1. Changes in the electrophoretic separation of 0.6 M KCl-soluble proteins during storage (A:unfrozen; B:stored at 30°C after 10 weeks storage; C:stored at a temperature fluctuating between -30 and -20°C at 3 day intervals; D:stored at a temperature fluctuating between -30 and -20°C at 7 day intervals; E:stored at a temperature fluctuating between -30 and -10°C at 3 day intervals; F: stored at a temperature fluctuating between -30 and -10°C at 7 day intervals.

The percentage of actomyosin in samples stored in temperature fluctuating between -30 and -20°C at 3 and 7 day intervals decreased gradually with the duration of storage (Figure 1-C, 1-D) and decreased to 30.93% and 25.10%, respectively, after 10 weeks storage. The percentage of actomyosin of samples stored at a temperature fluctuating between -30 and -20°C at 7 day intervals decreased faster than that of 3 day intervals.

As shown in Figure 1-E, 1-F, the percentages of actomyosin in samples stored with temperature fluctuating between -30 and -10°C at 3 and 7 day interval were 22.79% and 22.21%, respectively, after 10 weeks storage. The rate of decrease of actomyosin fraction in that of 3 day intervals of temperature fluctuation seemed more rapid than that of 7 day intervals of temperature fluctuation.

The changes in percentage of actomyosin calculated from the electrophoretic diagram were almost parallel to that of extractability of 0.6 M KCl soluble proteins and actomyosin. According to the above data, it is clear that the myofibrillar proteins deteriorated with the increase of temperature fluctuating amplitude. The percentage of unfrozen moisture in food can be calculated as the ratio of freezing

point to the body temperature /33/. The initial freezing point of mackerel is -1.5°C (conjectured from the freezing curve). Accordingly, the unfrozen moisture in frozen mackerel would be 5, 7.5 and 15% at -30 , -20 and -10°C respectively. During frozen storage, the unfrozen moisture would frequently change between 5% and 7.5%, or 5% and 15% when the samples stored with temperature fluctuating between -30 and -20°C , and between -30°C and -10°C , respectively. The unfrozen moisture in fish meat would cause the recrystallization and accretion of ice crystals during storage. In addition, the fluctuating temperature will enhance the recrystallization and accretion, because of the transfer of water molecules throughout the unfrozen solution /13,21/. Accordingly bonds among protein molecules which buttressed the tertiary structure of myofibrillar protein would be easily destroyed, and cause protein aggregation denaturation. The rate of recrystallization and accretion would be faster in samples stored with temperature fluctuating between -30 and -10°C than that of samples stored with a temperature fluctuating between -30 and -20°C . The degree of protein denaturation in larger fluctuating amplitude (-30 to -10°C) was, thus, higher than that of smaller fluctuating range one (-30 to -20°C).

Effect of temperature fluctuation on the gel-forming ability of frozen fish:

As shown in Table II, no significant difference in the folding test of Kamaboko was observed among the samples stored at -30°C and with temperature fluctuating between -30 and -20°C at 3 and 7 day intervals during 10 weeks storage, but it decreased in samples stored with temperature fluctuating at 7 day intervals after 10 weeks storage. No distinct change in gel strength which was based on the breaking force (g) and deformation (cm) was obtained from samples stored at -30°C during 10 weeks storage. However that of stored at temperature fluctuating between -30 and -20°C decreased gradually during 10 weeks storage. (from 1143 g.cm to 791 and 523 g.cm in samples stored with temperature fluctuating at 3 and 7 day intervals, respectively).

The Kamaboko gel-forming ability of samples stored with temperature fluctuating between -30 and -10°C decreased rapidly during 10 weeks storage (TABLE II). Although the folding test was still graded as "AA", the gel strength decreased from 1143 g.cm to 689 and 676 g.cm in samples with 3 and 7 day fluctuating intervals, respectively, after 6 weeks storage. These samples were not suitable for preparing a good quality of minced fish products after 10 weeks storage.

TABLE II
Effect of temperature fluctuation on the gelation of mackerel muscle during storage

storage time (wk)										
fresh	A*	B	6 C	D	E	A	B	10 C	D	E
BF**1120	1136	1134	1174	820	683	1040	1027	817	632	579
D** 1.02	0.99	0.93	0.90	0.84	0.99	0.82	0.77	0.64	0.73	0.67
GS**1143	1125	1055	1057	689	676	853	791	523	532	388
FD** AA	AA	AA	AA	AA	AA	AA	AA	A	A	B

* see TABLE I

** BF:breaking force (g); D:deformation (cm); GS:gel-strength (g.cm);
FD:folding test

It is obvious that the temperature fluctuation during storage do affect the quality of fish muscle, especially the gel-formation of muscle. The rate of deterioration in meat quality is contingent upon the amplitude and frequency of temperature fluctuation.

INFLUENCE DES FLUCTUATIONS DE TEMPERATURE SUR LA QUALITE DE LA CHAIR DE MAQUEREAU
(SCOMBER TAPEINOCEPHALUS) CONGELE.

RESUME : On a étudié l'influence d'une température fluctuante entre - 30 et - 20°C, - 30 et - 10°C à des intervalles de 3 et 7 jours sur la qualité (d'après l'extractibilité des protéines solubles dans 0,6 M de KCl et de l'actomyosine et la séparation électrophorétique des protéines solubles dans 0,6 M de KCl) et l'aptitude à former du gel de kamaboko (résistance du gel et essai de pliage) de maquereau congelé. Bien que la détérioration des protéines musculaires et de l'aptitude à former du gel de kamaboko des échantillons entreposés avec une fluctuation de température de - 30 à - 20°C à des intervalles de 3 et 7 jours fût légère au cours des 10 semaines d'entreposage, on n'a pas observé de différence entre ce groupe et les échantillons témoins (entreposés à une température constante de - 30°C). Cependant il se produisait une diminution de l'extractibilité des protéines solubles au sel, de l'extractibilité de l'actomyosine et de l'aptitude à former du gel de kamaboko dans les échantillons entreposés avec une fluctuation de température de - 30 à - 10°C à des intervalles de 3 et 7 jours au cours des 10 semaines d'entreposage.

REFERENCES

1. Hustrulid, A. and J.D. Winter: Agri. Eng., Vol.24 (1943) pp.416
2. Hustrulid, A., J.D. Winter and I. Noble: Refrig. Eng., Vol.57 (1949) pp.38
3. Pottinger, S.R.: Com. Fisheries Rev., Vol.13 (1951) pp.19
4. Kozima, T.: Cold Chain, Vol.2, No.3 (1976) pp.11
5. Kozima, T., S. Hotani, T. Mihiri and M. Hata: Bull. Intl. Inst. Refri., (1976) P.186
6. Nakamura, H.: Master's Thesis, Tokyo Univ. Fisheries, (1976)
7. Moleeratanond, W., B. W. Berry and W. Lee: J. Fd. Sci., Vol.45 (1981) pp.829
8. Sikorski, Z., J. Olley and S. Kostuch: Cri. Rev. Fd. Sci. Nutri., Vol.8, No.1 (1976) pp.97
9. Matsumoto, J. J.: In "Proteins at Low Temperature" (Ed. by O. Fennema), ACS Symp. Series 180, (1979) P.205
10. Matsumoto, J. J.: In "Chemical Deterioration of Proteins" (Ed. by Whitaker and Fujimaki), ACS Symp. Series 123, (1980) P.95
11. Shenouda, S. Y. K.: Adv. in Fd. Res. Vol.26 (1980) pp.275
12. Bramsnaes, F.: Fd. Technol. Vol.35 (1981) pp.38
13. Bevilacqua, A. E. and N. E. Zaritzky: J. Fd. Sci. Vol.47 (1982) pp.1410
14. Knooz, C. and J. M. Ramsbottom: Fd. Res. Vol.4 (1939) pp.117
15. Luyet, B. J. and M. C. Gibbs: Biodynamica Vol.1 (1973) PP.1
16. Lee, F. A., W. A. Gortner and J. Whitcombe: Fd. Technol. Vol.3 (1949) pp.164
17. Mazur, P.: Ann. N. Y. Acad. Sci. Vol. 85 (1960) pp.610
18. Luyet, B. J.: Ann. N. Y. Acad. Sci. Vol.85 (1960) PP.549
19. Luyet, B. J.: Recent Adv. in Fd. Sci. Vol.4 (1968) pp.247
20. Love, R. M.: J. Sci. Fd. Agri., Vol.13 (1962) pp.269
21. Sy, S. H. and O. Fennema: Proceedings Intl. Congr. Refri., Vol.3 (1973) pp.199
22. Sikorski, Z.: Int. J. Refri. Vol.1 (1978) pp.173
23. Sikorski, Z.: Adv. Fish. Sci. Technol. (Ed. by Connell, J.J.) Fishing News (Books), Ltd., England, (1980) pp.78
24. Noguchi, S.: New Fd. Industry Vol.21, No.3 (1982) pp.34
25. Noguchi, S.: Doctorate Dissertation, Sophia Univ., Tokyo, (1974) pp.12
26. Suzuki, T.: Fish and Krill Proteins, Processing Technology, Applied Science Pub., Ltd., London, (1981) pp.1-56
27. Horikuchi, T. and K. Hosotani: The Materials of the 16th Conference on Utilization and Processing Studies on Seafoods in Japan, (1982) pp.8
28. Itonami, A., M. Asahara and Y. Tanaka: ibid. (1982) pp. 15
29. Umemoto, S.: Bull. Jap. Soc. Sci. Fish., Vol.32 (1966) pp.427
30. Okada, M., M. Yokoseki and T. Nunomali: Minced Fish Meat Products, (Koseisha, Koseigaku, Tokyo), (1974) pp.374
31. Weber, K. and M. Osborn: J. Biol. Chem., Vol.244 (1969) pp.4406
32. Seki, N.: In "Shuisan Seibutsukagaku Shokuhin Zikensho", (by Saito et al., Koseisha, Tokyo), (1974) pp.124
33. Tanaka, K. and T. Kozima: Shokuhin Reito Kogaku (Japanese), Koseisha, Tokyo, (1976) pp.142

DISCUSSION

J.W. JEBSEN (Norway) - In the preparation of kamaboko from mackerel there remains a substantial quantity of offal. Does this offal have an influence on the properties of the kamaboko ?

SHANN-TZONG JIANG - I think the offal would affect the texture and whiteness of the kamaboko. However, the fish meat is subjected to refining processes and as a result we can say that the offal does not greatly influence the quality of the kamaboko product.

J.J. CONNELL (U.K.) - You compared the samples stored at fluctuating temperatures with those stored at constant temperature of -30°C . It seems to be that it would have been more appropriate to compare samples stored at fluctuating temperatures with samples stored at the equivalent temperature depending upon the change of reaction rate with temperature. The interesting point is whether fluctuating temperatures introduce any effect additional to that caused by storage at the equivalent temperature. Have you studied the second kind of comparison ?

SHANN-TZONG JIANG - No, but I appreciate your suggestion since it would be helpful to determine if there were any additional effects.

W.F.A. HORNER (U.K.) - 1) Has the folding tests been standardised ?

2) What are the dimensions of the slice taken ?

SHANN-TZONG JIANG - 1) The folding test and gel strength measurements are standardized methods for evaluating the quality of minced fish products in Japan.

2) The size of slice for the folding test is 3 mm in thickness and 30 mm in diameter.

DENATURATION OF SARCOPLASMIC PROTEINS DURING FROZEN STORAGE OF FISH

T. Børresen
L.B. Knudsen
J. Nielsen

Technological Laboratory, Danish Ministry of Fisheries
Technical University
Lyngby (Denmark)

1. INTRODUCTION

The sarcoplasmic protein fraction contains enzymes which are highly active post mortem, greatly influencing the quality of muscle foods during handling and storage. However, these proteins have received less attention than the myofibrillar proteins.

Early studies reviewed by Dyer and Dingle in 1961 /1/, indicated fish sarcoplasmic proteins to be little affected by frozen storage. No change in electrophoretic pattern could be detected after 2 months at -20°C /2/.

Later Connell in 1968 /3/ reviewed reports of changes in sarcoplasmic enzyme activities after frozen storage of fish muscle. It has since been shown by polyacrylamide gel electrophoresis that the sarcoplasmic protein pattern may be considerably changed after storage of fish at -15° and -20°C for 6 months /4/.

Isoelectric focusing (IEF) in thin gels represents a very accurate and highly reproducible method of protein detection, and IEF of sarcoplasmic proteins is now widely used for fish species identification /5/. In the present investigation, we have used IEF as a tool for studying changes in the sarcoplasmic protein fraction after frozen storage of fish.

2. MATERIALS AND METHODS

Fillets of post rigor cod (Gadus morhua), 40-60 cm in length, were minced in a meat chopper, and then thoroughly blended in a paddle mixer. The resulting mince was divided into 40 g portions which were vacuum packed and frozen at -10° , -20° , and -60°C .

Samples were taken out after 9 days, 44 days, and 10 months of storage. After thawing each sample was centrifuged for 20 minutes at 22,000 g in a refrigerated centrifuge (3°C). Samples of the centrifuged tissue fluid (CTF) were diluted 1:9 or 1:16 with distilled water (0°C) before IEF on agarose gels. CTF from one sample stored at -10°C for 10 months was diluted 1:2.

Agarose gels of 0.5 mm thickness were prepared by a procedure given by LKB /6/, with minor modifications, using LKB Isogel Agarose-EF 2206-111. For establishment of pH-gradient LKB Ampholine 1802-101, pH 4.0-6.5 was used. The electrofocusing was performed in a LKB 2117 Multiphor according to technical details given by LKB /6/.

After IEF the proteins in the gels were fixed in a solution of 100 mg/ml trichloroacetic acid and 10 mg/ml sulphosalicylic acid. The gels were then washed in 96% ethanol, stained in Coomassie Brilliant Blue R 250 (5 mg/ml), and destained in a solution of ethanol (33.6%) and acetic acid (3.6%).

The dried gels were scanned in a LKB 2202-002 Ultro Scan Laser Densitometer at a speed of 20 mm/min.

CTF from samples stored at -10°C and -60°C were filtered on a Sephadex G-100 column (Pharmacia C16/40) by elution with a 50 mM phosphate buffer, pH 6.7, at a flow rate of 0.19 ml/min. Fractions were pooled, and samples applied on agarose gels for IEF without sample dilution.

3. RESULTS

Isoelectric band patterns of sarcoplasmic proteins from samples stored at -10°C and -60°C are shown in Fig. 1. The sample stored at -60°C for 9 days serves as a reference. No changes were observed after further storage at this temperature. The band pattern of the sample stored at -10°C for 9 days is clearly different from the pattern of the -60°C sample. At -10°C there are more bands between band 2 and 3, and bands 3a and 7 are stronger than at -60°C . On the other hand, band 3 is weakened. The split seen in band 4 after 9 days at -10°C is assumed to be an artifact caused by sample application at this site.

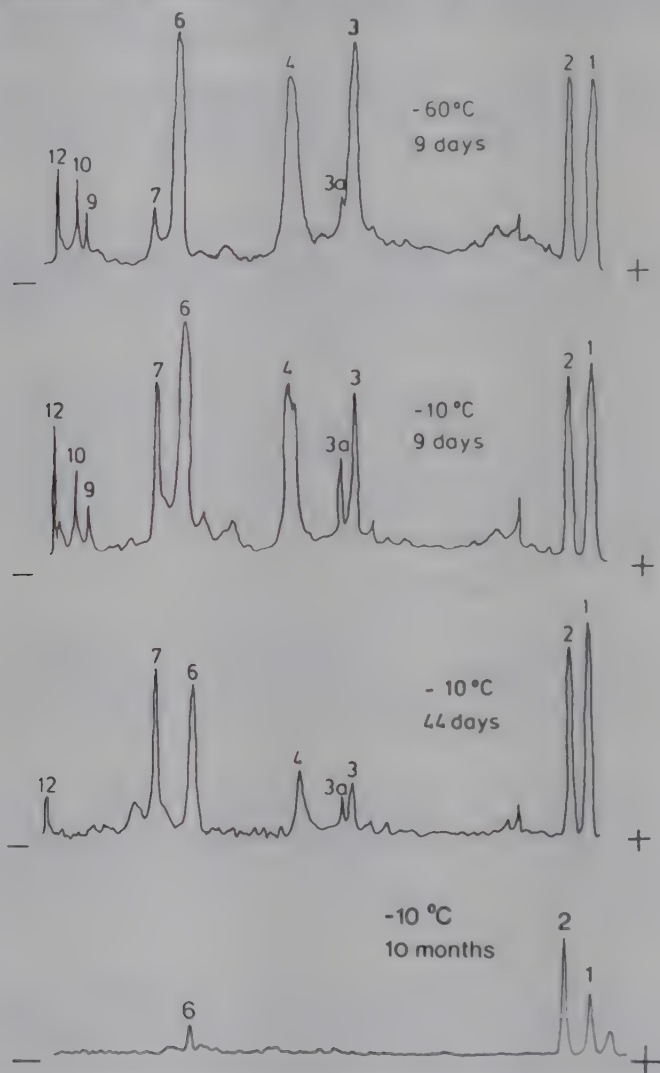


Fig. 1 - Isoelectric band pattern of sarcoplasmic proteins from fish muscle samples stored at -10°C and -60°C .

After 44 days storage at -10°C several bands have disappeared, and bands 3, 4, 6 and 12 considerably weakened. Band 7 seems to be unchanged from 9 days to 44 days storage, and band 3a is still pronounced. Band 1 and 2 appear to be constant.

After 10 months at -10°C only 4 bands can be observed. These are band 1, 2 and 6 in addition to a new, very anodic band.

In Fig. 2 the isoelectric protein bands from samples stored at -20°C are shown. After 9 days at this temperature a band pattern corresponding to the sample stored for 9 days at -10°C is seen, although bands 3a and 7 are less pronounced.

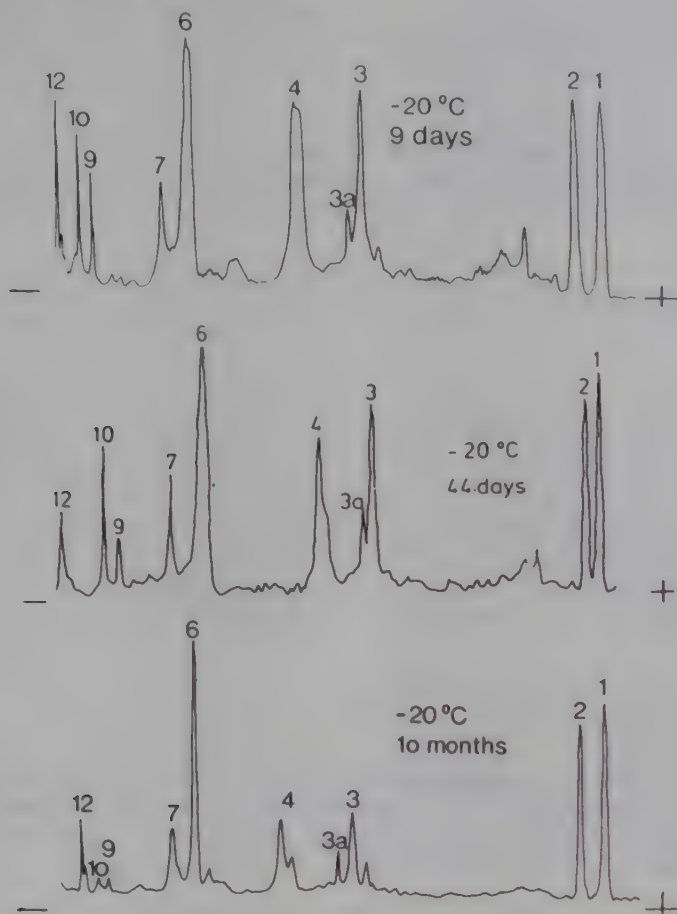


Fig. 2 - Isoelectric band pattern of sarcoplasmic proteins from fish muscle samples stored at -20°C .

After 44 days at -20°C it is noted that some bands may be a little weakened. Band 3a and 7 show the same stability as in the -10°C sample. After 10 months at -20°C all bands are considerably smaller, except bands 1, 2, 6 and possibly 7.

CTF from samples stored for 2 months at -20°C and -60°C were subjected to gel chromatography on a Sephadex G-100 column. The protein elution patterns are shown in Fig. 3.

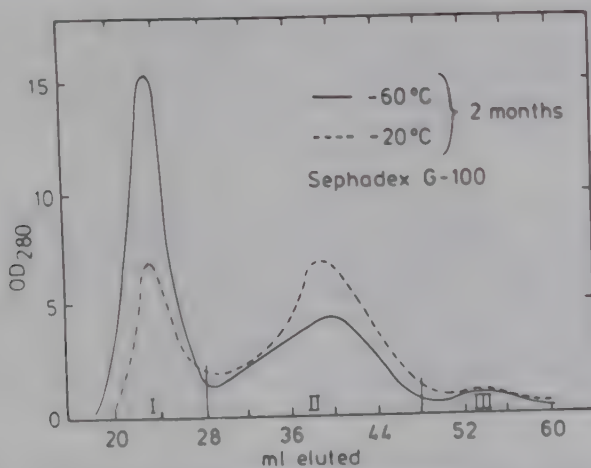


Fig. 3 - Gel chromatography of CTF samples from frozen fish muscle.

From a tentative calibration of the column, it is assumed that the first peak is representing proteins of MW >100,000. The second peak occurs at a position corresponding to a MW 50,000-60,000, and the third peak corresponding to a MW 10,000-15,000. The elution pattern indicates that there is a great difference in protein molecular weight distribution between the two samples. A larger proportion of proteins with high molecular weight is occurring at -60°C than at -20°C .

Samples from the three major fractions, I, II and III, respectively major fractions were applied on agarose gels, and the resulting IEF band patterns are shown in Fig. 4.

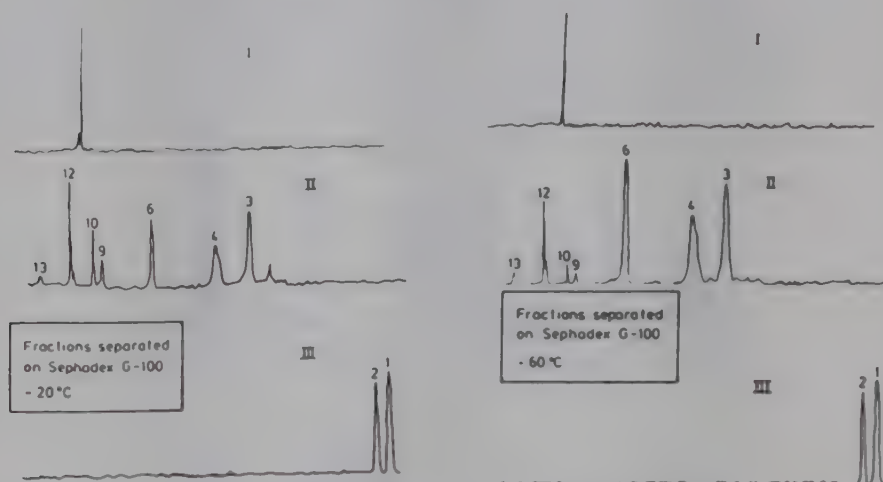


Fig. 4 - Isoelectric band pattern of sarcoplasmic proteins after separation on a Sephadex G-100 column. Fraction numbers corresponding to chromatography peaks in Fig. 3.

The very sharp peak occurring in the densitometer scans of fraction I from both samples does not represent an electrofocused protein, but indicates the point of sample application on the gel. This means that the proteins in fraction I have such a high molecular weight that they do not enter the agarose gel matrix.

From Fig. 4 it is further seen that the proteins representing bands 1 and 2 are separated into fraction III, while the remaining proteins are contained in fraction II. A very important exception is band 3a and 7, which cannot be detected in any of the samples. An interpretation is that the components representing bands 3a and 7 are of a very low molecular weight.

A further comparison of fraction II from the -20°C and -60°C samples seems to indicate that bands 3, 4 and 6 are weaker in the -20°C sample, and that bands 9, 10 and 12 are stronger.

4. DISCUSSION

The great success of using IEF of sarcoplasmic proteins for fish species identification, combined with the postulated great stability of this protein group, has led to the use of this method also for frozen samples /7/. However, changes in IEF of frozen samples have been detected, but were considered unimportant for species identification /8/. In the present work, the objective has been to further study these changes, and hence use IEF for studying protein denaturation during frozen storage.

The IEF technique provides a good tool for a general characterisation of a complex mixture of proteins, but accurate quantification of each protein is very difficult due to the variable diffusion and broadening of the components in the agarose gel. In connection with

species identification, a relative method of quantification has been worked out for estimating mixture ratios in samples containing more than one species /9/.

In the present work, no effort has been made to quantify the amounts of protein present, but as an estimate, band intensity may be compared with the intensities of band 1 and 2, which are the most stable during frozen storage. However, after prolonged storage at -10°C (Fig. 1) these proteins will also be affected by denaturation. The dry weight of the CTF from the sample stored at -10°C for 10 months has decreased by approximately 50% during storage, indicating a pronounced precipitative denaturation.

This development occurs both at -10° and -20°C , but at a much slower rate at -20°C . The general weakening of the protein bands is considered to reflect denaturation of proteins by aggregation and precipitation.

However, during the frozen storage, new bands are also occurring, indicating a change in isoelectric points for some components. This can be achieved by an aggregation of entities with different pI's, or by hydrolytic cleavage of smaller or larger parts of proteins. When aggregation takes place, and the molecular weight increases to a value substantially higher than 100,000, the aggregate will no longer enter the gel, as demonstrated in Figs 3 and 4. The new bands occurring after storage are therefore considered not to consist of aggregated proteins.

After gel filtration, the components representing the new bands can no longer be detected by IEF, as visualized in Fig. 4. These components may therefore be small fragments of proteins with molecular weight less than 10,000. An increase of these components after frozen storage indicates that proteolytic action must take place during freezing, in the frozen storage, during thawing, or immediately after thawing, as the time period between thawing and analysis is very short.

It is further observed that the amounts of the new components are highest at -10°C for 9 and 44 days. The initial high value could indicate proteolysis during freezing of the sample, which would progress most slowly at -10°C . It is, however, remarkable that the strength of band 7 remains unchanged together with bands 1 and 2 in the -10°C sample between 9 and 44 days, while all the other bands are decreasing. This could mean that the new component formed is very storage stable, or that new amounts are formed during storage. After 10 months at -10°C band 7 has disappeared together with the other bands, but at this stage denaturation is generally severe.

At -20°C band 7 is not as pronounced as at -10°C , and it is very difficult to detect any changes with storage time, whereas the other bands are showing a general decrease. This apparent stability of band 7, but increasing concentration initially at higher freezing temperature, (-10°C), may thus indicate that this component is formed by proteolytic action during freezing.

Changes in electrophoretic pattern of sarcoplasmic proteins in fish muscle after frozen storage which could be attributed to creation of low molecular weight components have previously been suggested by Umar and Qadri /4/, using polyacrylamide gel electrophoresis.

The presence of active, proteolytic enzymes, liberated from lysosomes during freezing, has been demonstrated by Rehbein et al. /10/, and suggested for use as a means of differentiating thawed and fresh fish fillets.

Any formation of new components during the period of frozen storage cannot be concluded from the present investigation, although Awad et al. /11/ after storage of fish at -10°C found that new elec-

trophoretic bands were formed after 16 weeks, using polyacrylamide gel electrophoresis.

None of the IEF bands have been identified so far. From the present study of IEF combined with gel filtration, it is, however, seen that bands 1 and 2 are very anodic and consists of protein with a low molecular weight ($\sim 15,000$). A survey of existing literature shows that fish muscle creatine kinase appears to have a pI around 4, and a MW $11,000 \pm 2,000$ /12/13/, indicating that band 1 or 2 could represent this enzyme, which also is known to be very stable toward precipitation in salt solutions /12/.

REFERENCES

1. W.J. DYER and J.R. DINGLE, Fish proteins with reference to freezing. In *Fish as Food* (G. Borgström, ed.), Vol. 1, Academic Press, London (1961), p. 275.
2. H.L. SEAGRAN, Analysis of the protein constituents of drip from thawed fish muscle, *Food Res.* 23 (1958) 143.
3. J.J. CONNELL, The effect of freezing and frozen storage on the proteins of fish muscle. In *low temperature biology of foodstuffs* (J. Hawthorn, ed.), Pergamon Press, Oxford, (1968) p. 333.
4. Z.N. UMAR and R.B. QADRI, Changes in the electrophoretic patterns of water soluble proteins of fish and shrimp during storage. *Pakistan J.Sci.Ind.Res.* 25 (1982) 176.
5. R.C. LUNDSTROM, Fish species identification by thin layer polyacrylamide gel isoelectric focusing: Collaborative study. *J. Assoc.Off.Anal.Chem.* 63 (1980) 69.
6. LKB 1818-A, Instruction for high-performance analytical electrophoresis in 0.5 mm thinlayer agarose gels.
7. J. YAMADA and A. SUZUKI, Identification of fish species by thin layer isoelectric focusing of sarcoplasmic protein. *Bull.Jap.Soc. Sci.Fish.* 48 (1982) 73.
8. J. GJERDE, Isoelectric focusing of water soluble fish protein as a means of fish species differentiation. *Fisk.Dir.Skr.,Ser. Ernæring*, 11 (1982) 45.
9. B. BØE, Quantitative separation of species in fish mixtures by multivariate analysis of electrofocused protein bands. *Food Chem.* 11 (1983) 127.
10. H. REHBEIN, G. KRESS and W. SCHREIBER, An enzymic method for differentiating thawed and fresh fish fillets. *J.Sci.Fd Agric.* 29 (1978) 1076.
11. A. AWAD, W.D. POWRIE and O. FENNEMA, Deterioration of freshwater whitefish muscle during frozen storage at -10°C . *J.Food Sci.* 34 (1969) 1.
12. K.S.P. RAO, B. FOCANT, C. GERDAY and G. HAMOIR, Low molecular weight albumins of cod white muscle (*Gadus callarias* L.). *Comp. Biochem. Physiol.* 30 (1969) 33.
13. R.K. SCOPES, Methods for starchgel electrophoresis of sarcoplasmic proteins. *Biochem. J.* 107 (1968) 139.

DENATURATION DES PROTEINES SARCOPLASMIQUES AU COURS DE L'ENTREPOSAGE DU POISSON A L'ETAT CONGELE

RESUME : La composition de la fraction de protéine sarcoplasmique du muscle de poisson varie continuellement au cours de l'entreposage à l'état congelé. Des dénaturations telles que l'augmentation de la concentration en sel, les variations du pH local, les forces mécaniques créées par la formation de cristaux de glace, etc, provoquent l'insolubilisation de certaines protéines, tandis que d'autres protéines initialement liées dans les organelles entrent dans un état de solubilité plus libre par suite de la déchirure des membranes. Des exemples de ce dernier type sont les enzymes métaboliques contenues dans les mitochondries et les enzymes protéolytiques du type cathepsine contenues dans les lysosomes.

Dans la présente recherche, les protéines et les peptides solubles dans une fraction de tissu centrifugé ont été étudiés après entreposage de muscle de cabillaud à l'état congelé. L'analyse par concentration iso-électrique des échantillons de fraction de tissu centrifugé a montré l'apparition de nouvelles bandes après 1 à 2 semaines seulement d'entreposage à -10°C . La séparation à la colonne Sephadex a indiqué que les nouveaux composants avaient une masse moléculaire inférieure à 10 000. On peut supposer que les composants proviennent de la protéolyse à l'état congelé. Un entreposage plus long donnait une proportion généralement moins grande de protéines dans les fractions de tissu, suivie d'un affaiblissement progressif de toutes les bandes de concentration iso-électrique.

Les transformations des protéines au cours de l'entreposage à -20°C progressent plus lentement qu'à -10°C , mais la configuration de la transformation est, avec quelques différences, comparable aux deux températures.

DISCUSSION

I.M. MACKIE (U.K.) - Your observations on the decrease in the extractability of the sarcoplasmic proteins on frozen storage are in some conflict with present views on their stability. Would you care to comment on the use of the well established electrophoretic method of identification of unfrozen and frozen stored fish in the light of your findings since the method is dependent on the species - specific patterns of sarcoplasmic proteins being unaffected by the frozen storage conditions of fish ?

T. BØRRESEN - I don't think the changes caused by frozen storage will affect the band pattern so much that it will confuse species identification by isoelectric focusing on frozen samples. The changes we have demonstrated are in severely denatured fish. Under 'normal' circumstances with short freezing times and low storage temperatures, only small differences in band patterns are observed.

H. REHBEIN (FRG) - In which way was the sarcoplasmic fraction prepared ?

T. BØRRESEN - Either as centrifuged tissue fluid (CTF) or as an extract. The IEF results were the same with both methods.

LIPID CHANGES IN COD MUSCLE TISSUE FROZEN IN LIQUID NITROGEN DURING FROZEN STORAGE

L. STODOLNIK

Institute of Marine Food Technology, Faculty of Sea Fisheries and Food Technology, Academy of Agriculture, Szczecin (Poland)

1. INTRODUCTION

The contents of lipids in fish muscle tissue is mainly conditioned by genetic characters. Lipids occur in subcutaneous layer, between muscle fibres, in myosepts and as components of cells. Their location and composition depend on the total lipid contents /1/. In fat fish, lipids constitute an energetic reserve substance, mainly as triglycerides in the subcutaneous part and between muscle fibres; in lean fish, they occur mainly as phospholipids in muscle fibres and as individual cells /2, 3/.

The amount of lipids, their location and composition influence on the final results of crystallization process in frozen fish. In lean fish the negative effects of crystallization process are greater than in fat ones /4/. The tissue sap crystallization in lean raw materials results primarily in structural changes of cellular membranes due to reduced energy of hydrophobic bonds in lipoproteids. Consequently the sarcolemma may become loosened and some of its lipid components may get more susceptible to extraction with organic solvents and to oxidation. Anterior studies showed the cholesterol release from membrane structural components due to freezing /5,6/. Studies of Toyomizu /7/ suggest the importance of crystallization in fish flesh for accelerating the glycerylphosphorylcholine accumulation during frozen storage.

The susceptibility to oxidation of protein-bound components of lipids in muscle tissue during the freezing preservation has not been explained.

The dimensions of changes resulting from crystallization depends on conditions of freezing and storage. In the individual technology of fish freezing, chiefly in the cryogenic one, the temperature along the fish body varies due to varying thickness of the body. Therefore, the fish lipids in frozen storage may be affected by the final temperature attained after freezing.

The present work analyses the amount of total and free lipids, fatty acids, and extent of oxidation of cod fillets, liquid nitrogen-frozen to 263K, 243K, and 213K and stored at 245K.

2. MATERIAL AND METHODS

The assays were made on the Baltic cod /*Gadus morhua* L./ processed to fillets on Baader 188. The fillets of a similar thickness /about 22 mm/ were cut from the cephalic part and examined. They were past the rigor mortis stage. The fillets were individually frozen in liquid nitrogen in a British Oxygen Company laboratory tunnel. The freezing temperature in the nitrogen injection ranged within 138K-133K. The time of freezing to the final temperature of 263K, 243K and 213K in the fillet thermal centre was 8.5, 10.5 and 12.5 min., respectively. The frozen fillets were glazed with tap water ice-cooled to about 273K. Each fillet was then wrapped in 0.015 mm thick aluminium foil and placed in standard frozen fish cartons. The raw materials were stored for 12 months at 245K. The fillets were thawed in polythene bags under water shower of 291-293K to 272-273K in the thermal centre. From each experimental batch 15 fillets were taken for the analysis. The fillets were skinned, dorsal parts of white muscles were cut out and minced.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

The following analytical techniques were used: total lipid extraction with chloroform-methanol /8/, free lipid extraction with carbon tetrachloride /4/; free fatty acids, phospholipids and triglycerides were analysed by thin layer chromatography /hexane:ethyl ether:acetic acid/, their content being determined densitometrically, after charring, at 450 nm wave length relative to oleic acid, lecithin and tripalmitin+triolein. Lipid oxidation was determined rodanometrically /9/.

3. RESULTS AND DISCUSSION

The cod muscle lipid content analysis showed a low amount of free lipids to be present /0.08%/. The decidedly higher content of chloroform-methanol extracted lipids shows the cod muscle lipids to be confined mainly to muscle cells; these lipids make up about 83% of the total lipids extracted /Fig.1/. The main components of total and free lipids were phospholipids making up about 61% and 73% respectively /Table I/.

TABLE I
Phospholipids and triglycerides content in cod lipids

Lipid fractions	Before freezing		After 12 months of storage					
	total lipids	free lipids	total lipids			free lipids		
			263K	243K	213K	263K	243K	213K
Phospholipids	60.8	73.5	70.9	67.6	55.8	48.6	33.5	52.5
Triglycerides	8.9	5.6	5.1	5.2	10.0	5.4	4.2	13.0

Changes in lipid susceptibility to organic solvents were detected during the freezing storage. Those lipids occurring in fillets frozen to 213K varied most, while the smallest changes occurred in the flesh frozen to 263K /Fig.1/.

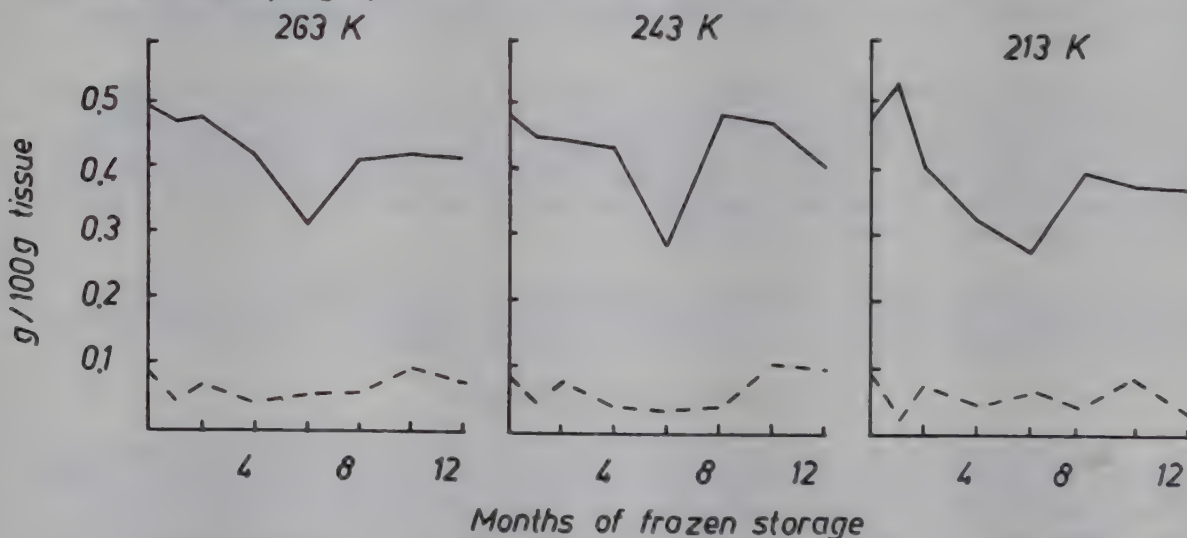


Fig. 1 - The influence of the final temperature /263K, 243K, 213K/ on free - - - and total — lipid contents in cod muscle tissue during storage at 245K

The free fatty acids content in cod lipids increased over the frozen storage period, the increase being faster in the total lipid fraction /Fig. 2/. Changes of this nature point out to the prevalence of destruction in unstable lipid-protein bindings over phospholipid and triglycerides hydrolysis in frozen-stored cod flesh /Fig. 3/. The dynamics of FFA content in total and free lipids, as expressed by the ratio of those components, over the cod fillets frozen storage period support the notion of bound lipids being the FFA source during freezing and frozen storage of cod, particularly the fillets frozen to 263K and 213K /Fig. 4 /.

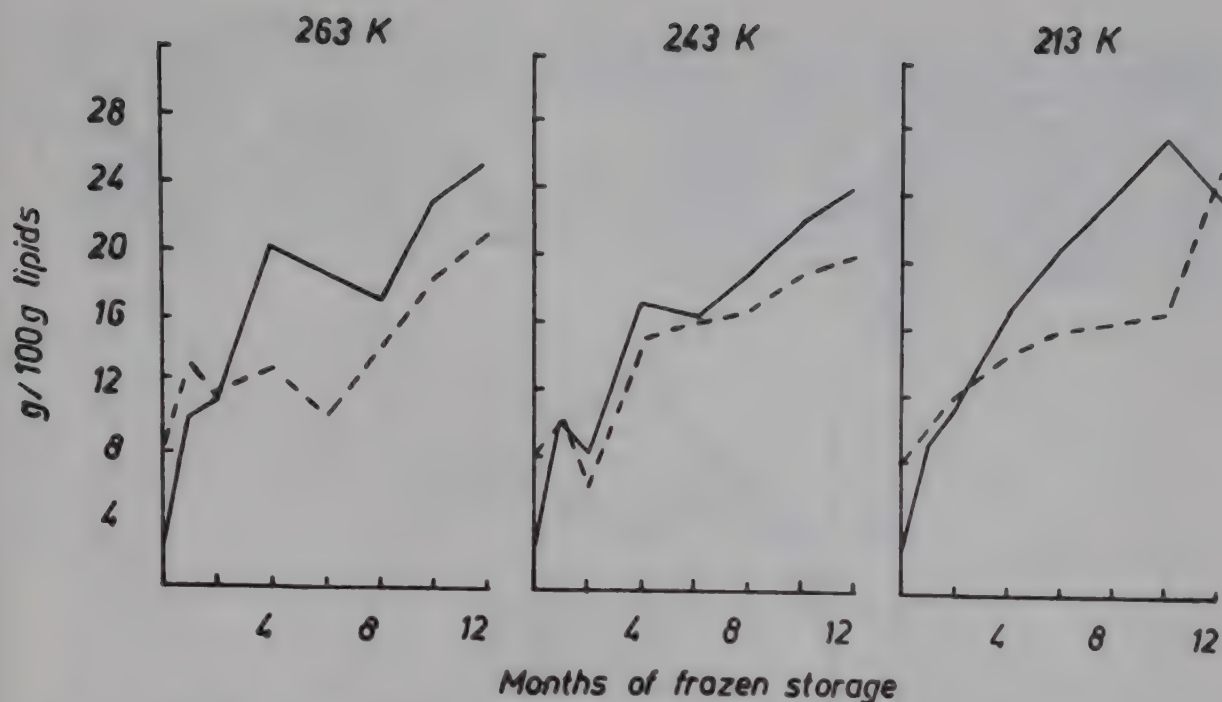


Fig. 2 - The influence of the final temperature on free fatty acids content in cod lipids during storage at 245K, - - - in free lipids, — in total lipids

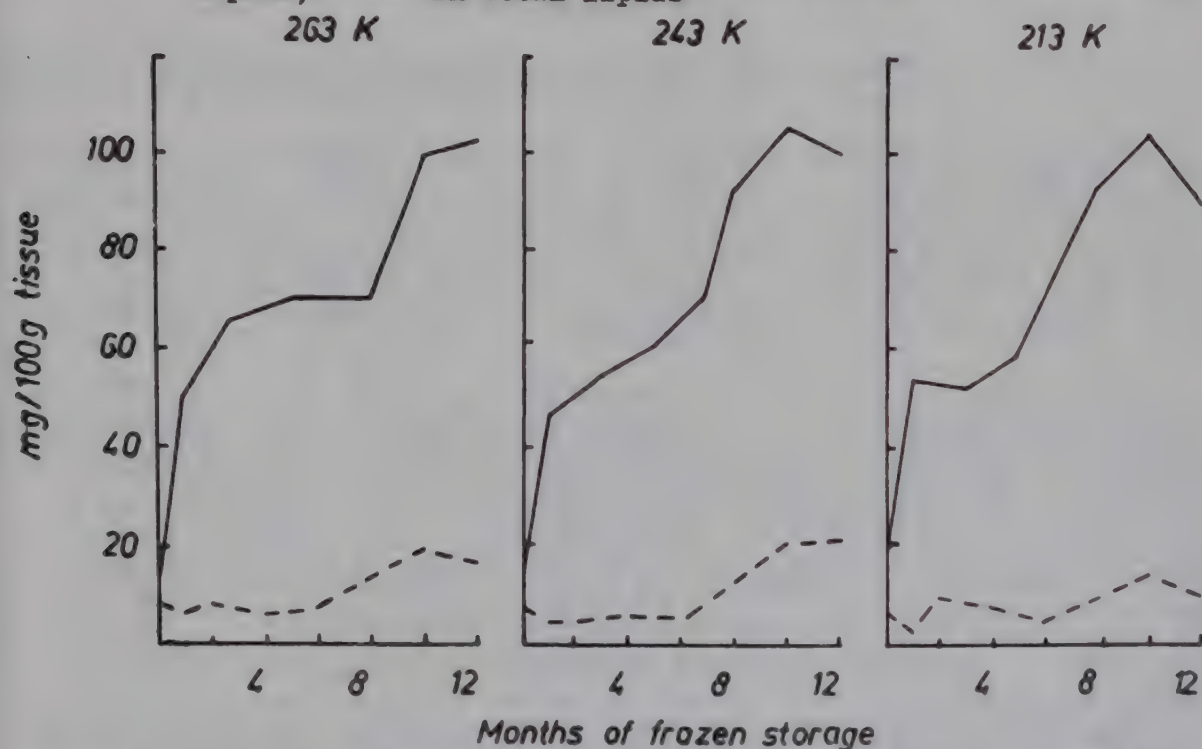


Fig. 3 - Free fatty acids content in cod muscle tissue accounted for free - - - and — total lipids, during storage at 245K

Changes in the FFA contents proceeded with a varying rate throughout the period of cod frozen storage. During the initial few weeks, the FFA content was found to increase rapidly; the increase showed down between the 4th and 8th month to grow rapidly again further on /Fig. 2/. The effects of the final temperatures /263K and 243K/ on fatty acid release during storage were similar, the temperature of 213K contributing to the highest FFA content in free lipids during the terminal period of storage. The high FFA contents /21% - 26%/ and the reduced amount of phospholipids in free lipids /Fig. 2. Table I/ after 12 months of cod

fillets storage at 245K may also evidence the activity of hydrolytic enzymes at low temperatures. Considering the quantitative changes in FFA and phospholipids during the frozen storage of cod fillets, the lipolytic enzymes may be suspected to be more active after about a half-year storage at 245K. The lipid hydrolysis rate might have changed due to a mechanical damage of lysosomes and mitochondria during prolonged frozen storage, resulting both in the lipids becoming more accessible and enzymes more concentrated. Moreover, the enzymes might have been transformed into more active forms.

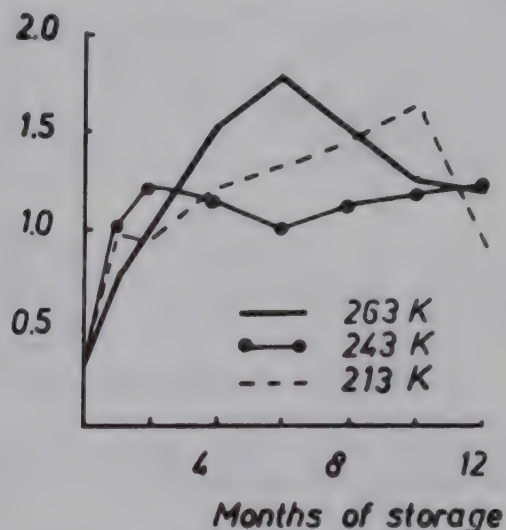


Fig. 4 - The ratio of free fatty acid contents in total and free lipids during storage of cod at 245K

When the FFA content dynamics is compared with that of total lipids over the entire period of cod fillets storage /Fig.2,1/, one can see that in spite of a reduced content of the extracted lipids between the second and sixth month of storage, their FFA content tended to increase, which may indicate a more stable interaction between proteins and lipid components other than fatty acids at some stages of frozen storage. Other studies /10/ showed phospholipids, triglycerides and cholesterol to be the most reactive lipid components in cod fillets stored frozen.

The fatty acid release was accompanied by lipid oxidation. The peroxide content in free lipids was higher than that in total lipids /Fig.5/. The highest peroxide content was found in cod fillets after 6 months of storage. At that time, free lipids of the cod muscles frozen to 213K contained 4 times as high peroxide content as that in total lipids; in the fillets frozen to 263K and 243K, the free lipids peroxide content differed from that in total lipids by the factors of about 7 and more than 17, respectively. After the 6 month of storage on, the peroxide content was found to drop in all types of material tested. At that time, FFA contributed more than 20%. Such amounts of free fatty acids might have acted as inhibitors in lipid oxidation under frozen storage conditions /11/. Over the entire period of frozen storage, free lipids of the fillets frozen to 213K were the least oxidated group, presumably due to a higher nitrogen absorption on freezing in this material than in the fillets frozen to 243K and 263K. Other studies /6/ show that during the cod fillets frozen storage, both free fatty acids and esthers are oxidated. Free fatty acids in cod lipids were subject to the slowest oxidation among the lipid components.

The cod flesh peroxide content calculated /Fig. 6/ showed the amounts to be similar when the total and free lipid oxidation was accounted for / or a higher amount in the latter case/ which allows to conclude that they were those lipid components occurring as free in the cod muscle tissue that were oxidated. On the other hand, lipids in unstable binding with protein were not subject to oxidation. Presumably protein in the lipoprotein molecule protects lipids from oxidation. Such a sug-

gestion may be based on other studies /12/ which point out to some anti-oxidative properties of protein of certain fish treated thermically.

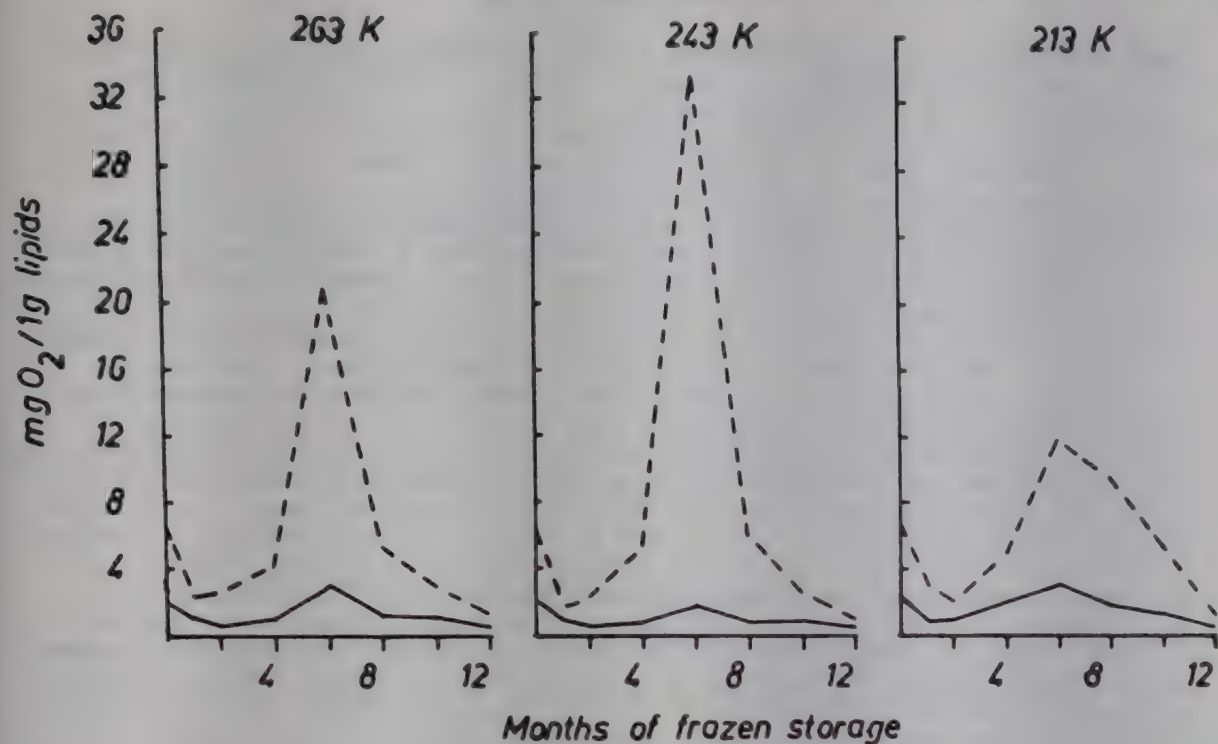


Fig. 5 - Peroxide contents in cod lipids during storage at 245K, --- in free lipids, — in total lipids

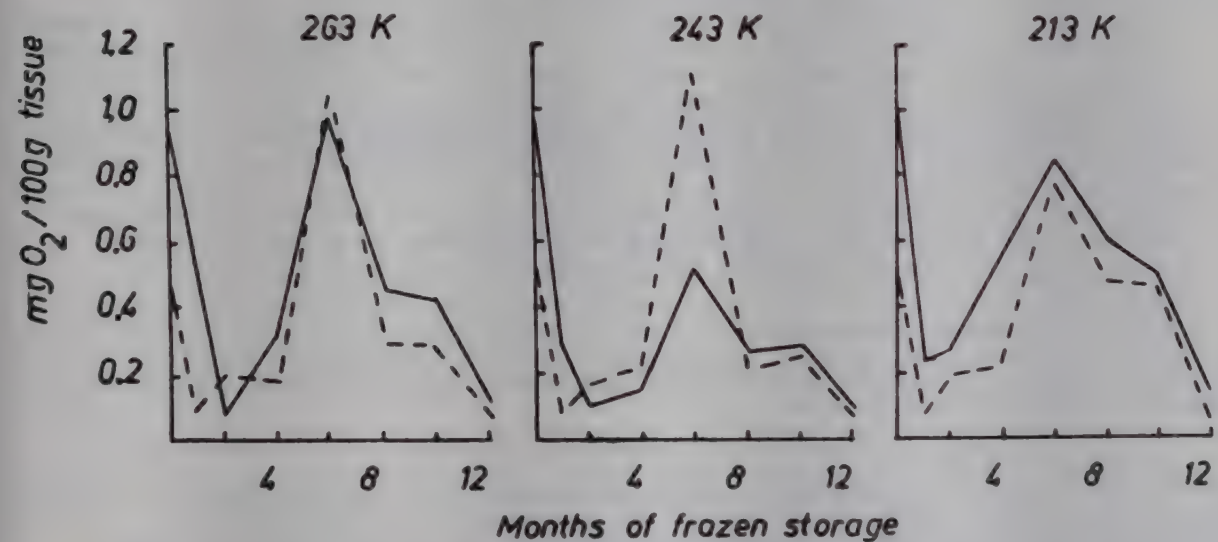


Fig. 6 - Peroxide contents in cod muscle tissue accounted for free --- and total — lipids, during storage at 245K

The analysis of lipid compounds in cod fillets frozen in liquid nitrogen showed the final temperatures above and below the temperature of storage act in favour of the release of fatty acids from cell membrane lipoproteids. Consequently, fatty acids are lipid compounds having the weakest bonds with proteins. Structural changes in cell membranes brought about by crystallization may affect the cod flesh texture by way of increasing its plastic properties /10/.

Changes in lipids of cod flesh frozen in liquid nitrogen and stored for 12 months at 245K prove the fillets can be frozen under such conditions to temperatures much higher /263K/ than the temperature of storage.

REFERENCES

1. I.F. LEVANIDOV, L.P. BACHOŁDINA, G.N. RYBOŁKINA: Lipidy myszecznoej tkani ruvietty / Lipids of muscle tissue of Ruvettus pretiosus/. Rybna Choz., n.2 /1979/ pp. 60-62.
2. J.E. KINSELLA, J.L. SHIMP, J. MAI: The proximate and lipid composition of several species of freshwater fishes. New York's Food and Life Sciences Bull., n. 69 /1978/ pp.1-20.
3. Z. PODESZEWSKI, L. STODOLNIK: Lipidy przemysłowych ryb bałtyckich, VII. Zmiany frakcji lipidowych ryb bałtyckich w cyklu rocznym /Changes in lipid fraction of fish at year cycle/. Bromat.Chem.Toksykol., Vol. 9 /1976/ pp. 341-347.
4. Z. PODESZEWSKI, L. STODOLNIK, M. KNASIAK, J. ŚWINIARSKA: Ocena wpływu zamrażania LN₂ na niektóre właściwości konsumpcyjne i przetwórcze ryb /Evaluation of liquid nitrogen frozen fish/. Academy of Agriculture, Szczecin, /1977/ /typescript/.
5. R.V. KRISHNAMOORTHY, A. VENKATARAMIAH, G.J. IAKSHMI, P. BIESIOT: Effects of cooking and of frozen storage on the cholesterol content of selected shellfish. J. Food Sci., Vol. 44 /1979/ pp. 314-315.
6. L. STODOLNIK: Przemiany lipidów ryb w czasie zamrażalniczego przechowywania /Changes of fish lipids during frozen storage/. Publication-Academy of Agriculture, Szczecin, Dissertations No 76 /1981/.
7. M. TOYOMIZU, K. HANAOKA, K. SATAKE, H. NAKAGAWA: Effects of storage temperatures on accumulation of glycerylphosphorylcholine and decomposition of phosphatidylcholine in fish muscle during cold storage. Bull. Japan. Soc. Sci. Fish., Vol. 43 /1977/ pp. 1184-1187.
8. RR. LINKO: Ann. Univ. Turkuensis. AI /1967/, pp. 21.
9. C. PIETRZYK: Kolorymetryczne oznaczanie nadtlenków w tłuszczach za pomocą rodanków żelaza /Colorimetric determination of peroxides in fats/. Roczniki PZH, Vol. 9 /1958/ pp. 76-84.
10. L. STODOLNIK, M. KNASIAK: Zmiany ilości lipidów wolnych i związanych i ich wpływ na konsystencję tkanki mięśniowej dorsza w czasie zamrażalniczego przechowywania / Changes in free and connected lipid contents and their influence on consystence of cod muscle tissue during frozen storage/. Przem. Spoż., n. 12 /1984/.
11. C.H. CASTELL, B.A. MOORE, P.M. JANGAARD, W.E. NEAL: Oxidative rancidity in frozen stored cod fillets. J. Fish. Res. Bd. Canada, Vol. 23/1966/ pp. 1385-1401.
12. D.P. SEN, C.S. BHANDARY: Lipid oxidation raw and cooked oil sardine /Sardinella langiceps/ fish during refrigerated storage. Lebensm.-Wiss. u.-Technol., n. 11/1978/ pp. 124-127.

MODIFICATIONS DES LIPIDES DU TISSU MUSCULAIRE DE CABILLAUD CONGELE DANS L'AZOTE LIQUIDE AU COURS DE L'ENTREPOSAGE.

RESUME : On a déterminé l'influence d'une température finale de 263K, 243K et 213K de filets de cabillaud refroidis par des aspersion à N... liquide sur la quantité des lipides libres et totaux, la rapidité de leur oxydation et la teneur en acides gras libres au cours de l'entreposage à 245K.

Les expériences ont montré que les lipides libres constituaient environ 15 % des lipides totaux du tissu musculaire de cabillaud et que les phospholipides étaient le constituant dominant dans les deux formes de lipides.

On a constaté, au cours de l'entreposage, une augmentation rapide de la quantité des acides gras libres dans les lipides totaux, mais une augmentation plus intense des lipides totaux, en particulier dans les filets congelés à 213K et 263K. On a constaté, en même temps, que ce n'était que les lipides libres qui subissaient l'oxydation, et que la quantité de peroxydes dans les lipides totaux du tissu musculaire de cabillaud était due à l'oxydation des constituants lipidiques présents sous forme libre. La matière première congelée jusqu'à la température finale la plus basse subissait la moins forte oxydation au cours de l'entreposage.

CHANGES IN FISH MUSCLE COLLAGEN DURING FROZEN STORAGE

A.J. Borderías
P. Montero
Instituto del Frío, Madrid (Spain)

1. INTRODUCTION

A number of authors have related texture changes taking place during frozen storage to aggregation of the myofibrillar proteins (Dyer and Dingle /1/; Connell /2/; Sikorski /3/; etc.). Nevertheless, certain others (Bremner /4/; Bremner et al. /5/; Sikorski /6/) have reported that, in addition to changes in the myofibrillar proteins, other unspecified factors also play a role.

Based on Tran's /7/ idea that the texture of frozen fish muscle may be altered by break-down of the bonds of the collagen, and assuming that the hardening of the sarcolemma detected by Love /8/ and Tanaka /9/ might be due to the surrounding layers of endomysium, muscle collagen was hypothesized to be a source of hardening in frozen fish muscle.

The purpose of the present study, then, was to determine alterations in the state of the frozen fish muscle collagen and the inter-molecular bonds in two species with different texture behaviour during frozen storage, i.e., hake and trout. If the results suggested that changes do take place in the collagen, subsequent research would focus on collagen's contribution to alterations in texture and on ways of solving the problem.

2. MATERIALS AND METHODS

Preparation of samples

Trout (Salmo irideus Gibb) and hake (Merluccius merluccius L.) were used. A homogeneous lot of 9 kg of trout was obtained from a fish farm; mean length was 26.79 cm and mean weight 310 g. The fish were killed by breaking their spines, and the experiment got under way 16 hours later.

The hake were caught by long-line on the Galician shelf in two different months, June and October. For both months homogeneous, 15-kg lots were used; mean length was 57.5 cm and mean weight 1.6 kg. The experiment began 24 hours after capture in the case of hake, after rigor mortis had subsided.

The muscle from trout (T) and from October hake (H-18) was skinned and cut into pieces. The hake caught in June (H-12) was minced in a Baader 694 deboner using an orifice size of 3 mm.

The samples, prepared as described above, were vacuum-bagged in 200-g portions in cryovac BB-1 plastic pouches and frozen in a blast freezer. Storage was effected at -18 °C for lot H-18 and at -12 °C for lots T and H-12.

Proximate analysis

Following AOAC /10/ standards.

Routine analyses

Storage time was 160 days for lot H-12, 120 days for lot H-18, and 90 days for lot T. The lots were tested four or five times during

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom)- 1985-4

this period, and the tests consisted of the following analyses:

- Separation and purification of the connective tissue, as set out below in Figure 1. The yield of connective tissue on separation from the muscle was around 99 %;

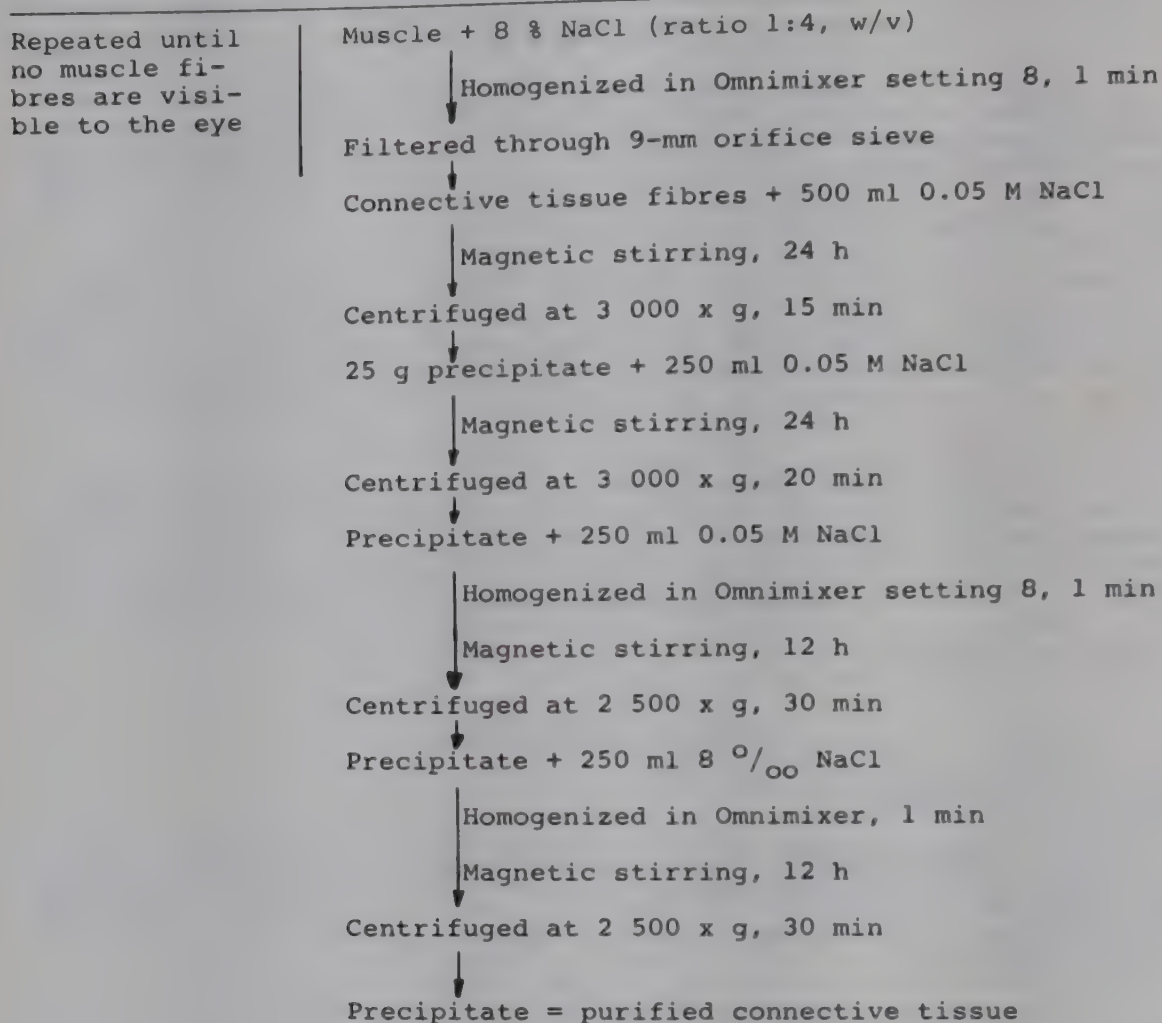


Fig. 1 - Separation and purification of connective tissue

- Analysis of hydroxyproline following the method of Leach /11/;
- Solubilization of the collagen using a modified method of Timpl et al. /12/, as shown in Figure 2;
- Analysis of the α , β , and γ components of acid-soluble collagen by molecular sieve chromatography according to a method based on the techniques of Chandakasan et al. /13/ and Krieg et al. /14/, as shown in Figure 3.

Statistical analysis

The standard deviation was calculated for the mean values obtained in each analysis.

3. RESULTS AND DISCUSSION

Proximate analysis

Table I gives the proximate composition of the muscle tissue

employed in the samples.

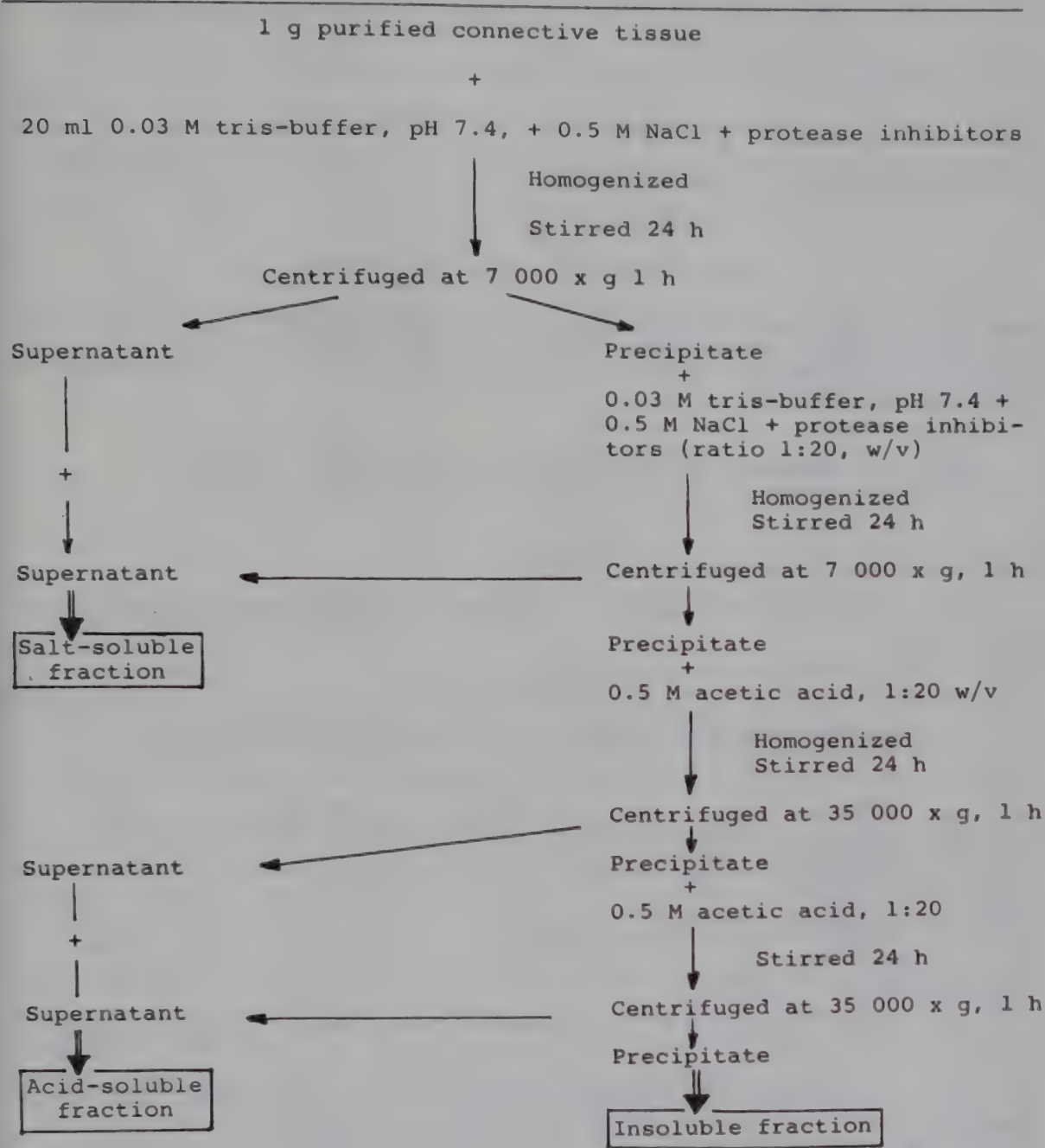


Fig. 2 - Collagen solubilization

TABLE I

Proximate analysis and initial pH of the fish muscle

	H-12	H-18	T
Moisture	82.11 ± 0.1	81.80 ± 0.6	76.30 ± 0.8
Ash	1.24 ± 0.1	1.04 ± 0.1	1.21 ± 0.0
Crude fat	1.62 ± 0.1	0.45 ± 0.0	5.06 ± 0.5
Crude protein	17.21 ± 0.2	18.03 ± 0.8	17.78 ± 1.5
pH	6.90 ± 0.0	6.99 ± 0.0	6.43 ± 0.0

Acid-soluble collagen + 2.7 M NaCl

↓ Centrifuged at 35 000 x g, 1 h

Precipitate + 0.5 M acetic acid (concentration 4 mg/ml)

↓

Dialyzed against 0.1 M acetic acid

↓

Dialyzed against 50 mM tris-buffer, pH 7.5 + 1 M CaCl₂ + 0.02 % azide

↓

Denatured at 60 °C, 30 min

↓

Centrifuged at 3 000 x g, 20 min

↓

Supernatant analyzed in a Biogel A 5.0 m chromatography column 1.5 m long by 2.1 cm in diameter. Buffer: 50 mM tris-buffer, pH 7.5 + 1 M CaCl₂ + 0.02 % sodium azide. Constant flow of 30.1 ml/h. Absorbance determined at 220 nm.

FIG. 3 - Analysis of α , β , and γ components of collagen

Yield of separated connective tissue

Table II shows the yield of connective tissue from muscle after purification.

Table II

Yield of connective tissue from muscle (% dry matter)

Ratio	H-12	H-18	T
Separated connective tissue: muscle	2.91 ± 0.2	3.09 ± 0.3	1.33 ± 0.0
Purified connective tissue: muscle	1.75 ± 0.1	1.76 ± 0.2	1.17 ± 0.0

The above figures are in agreement with those given by Jacquot /15/, who states that connective tissue makes up about 2-5 % of the muscle in teleosts. However, after purification the proportion shown in Table II was somewhat lower, probably due to the fact that some components of the connective tissue, such as polysaccharides, lipids, etc. were removed along with the residual myofibrillar proteins. There was no hydroxyproline loss during purification, hence there was a proportional enrichment of collagen in the purified sample (H-12 = 66.6 %; H-18 = 62.5 %; T = 14.3 %).

According to Love /16/, reporting studies performed on cod, a species phylogenetically similar to hake, the connective tissue, in particular the insoluble fractions, increases in undernourished fish. However, the data in Table II indicate almost no variation in the proportion of connective tissue in the muscle of fish of the same species in different nutritional condition (H-12 and H-18).

Collagen solubility

The data on collagen solubility over the storage period appear in Table III.

From the data for day 0 (unfrozen sample) the evidence is insufficient to support the theory of Love /16/ and McBride et al. /17/

TABLE III

Proportion of collagen fractions in the samples over storage

Sample	Days	Fraction (%)		
		SS	AS	I
H-12	0	8.4 $\pm$ 0.5	83.5 $\pm$ 0.9	8.1 $\pm$ 0.0
	25	11.3 $\pm$ 0.2	41.7 $\pm$ 0.8	47.0 $\pm$ 2.9
	70	1.2 $\pm$ 0.3	14.4 $\pm$ 2.1	81.4 $\pm$ 3.8
	160	1.6 $\pm$ 0.0	20.5 $\pm$ 2.1	77.9 $\pm$ 7.1
H-18	0	3.9 $\pm$ 0.1	89.9 $\pm$ 4.6	6.2 $\pm$ 0.1
	40	4.1 $\pm$ 0.3	76.3 $\pm$ 6.8	19.6 $\pm$ 0.6
	70	7.2 $\pm$ 0.1	52.1 $\pm$ 0.7	40.7 $\pm$ 0.5
	120	3.8 $\pm$ 0.4	64.2 $\pm$ 0.8	32.0 $\pm$ 0.9
T	0	2.3 $\pm$ 0.1	71.5 $\pm$ 0.5	26.2 $\pm$ 0.9
	25	3.1 $\pm$ 0.5	75.6 $\pm$ 0.8	21.4 $\pm$ 1.9
	40	1.9 $\pm$ 0.0	82.0 $\pm$ 7.7	16.0 $\pm$ 0.0
	70	6.7 $\pm$ 0.2	75.8 $\pm$ 2.1	17.5 $\pm$ 1.3
	90	3.3 $\pm$ 0.0	74.4 $\pm$ 0.3	22.9 $\pm$ 0.2

SS: salt-soluble fraction; AS: acid-soluble fraction;
I: insoluble fraction

that the collagen of hake in better nutritional condition (H-12, from the proximate analysis shown in Table I) is more soluble than that of less well-fed hake (H-18). It can also be observed that the proportion of insoluble collagen is higher in trout than in hake, which may help to explain the firmer texture of trout.

Turning to the changes taking place in muscle collagen over the storage period, the collagen from sample H-12 became more insoluble with time than did the collagen from sample H-18, suggesting that the higher storage temperature and/or mincing (H-12) exerted some influence on the degree of insolubility. There were only slight variations in sample T during storage. The fact that trout collagen behaved differently than hake collagen under freezing may have been due not only to its distinct nature but also to the action of various substances intrinsic to the different muscles with a denaturing or preservative effect.

α , β , and γ components of acid-soluble muscle collagen

The data from analysis of the α , β , and γ components of collagen over the storage period are presented below in Table IV.

Looking at day 0, that is, the fresh sample, it can be noted that the greater abundance of α monomers and lesser abundance of γ trimers in the better nourished H-12 lot supports the hypothesis that collagen from undernourished fish has more cross-links (Ironsides and Love /18/; Asghar and Henrickson /19/). Nonetheless, in the present case such cross-links were not complex enough to be reflected in changes in the solubility, as previously stated.

The fact that a higher proportion of α components and no γ components were detected in the acid-soluble trout collagen, plus the large amount of insoluble collagen in fresh trout (Table III), may be due to the said γ components being composed of highly reduced, stable cross-links, causing them to form part of the insoluble collagen fraction rather than of the acid-soluble collagen (Sims and Bailey /20/).

The analysis of the α , β , and γ components during storage showed that the proportion of the α components decreased over the storage

TABLE IV

Proportion of α , β , and γ components in acid-soluble collagen in the different samples over the storage period

Sample	Days	Components (%)		
		α	β	γ
H-12	0	36.7	49.3	18.0
	25	11.2	63.6	25.2
	70	10.2	61.1	28.7
	160	17.3	82.7	
H-18	0	3.5	66.2	30.3
	40	2.4	60.6	37.0
	70	0	57.3	42.7
	120	0	55.2	47.7
T	0	72.6	27.4	0
	25	55.1	44.9	0
	70	9.0	75.2	15.8
	90	5.2	63.7	31.0

period, changing into components having a larger number of cross-links, with the β dimers, the least complex, predominating. When the said cross-links were sufficiently strong, forming highly reduced, complex structures, they became part of the insoluble fraction. In the trout sample there was an increase in the number of cross-links between chains, with larger amounts of the β and γ components occurring with time, though the γ components were never made up of sufficiently complex cross-links to result in insolubilization of the collagen.

BIBLIOGRAPHY

1. W.J. DYER and J.R. DINGLE, Fish proteins with special reference to freezing. In "Fish as Food", vol. 1, G. BORGSTROM (ed.). Academic Press, New York (1961), p. 275.
2. J.J. CONNELL, Fish muscle proteins and some effects of processing on them. In "Proteins and their Reactions", SCHULTZ and ANGLEMIER (eds.). AVI, Westport, Conn. (1964), p. 255.
3. Z.E. SIKORSKI, Technologia Żywności Pochodzenia Morskiego (Marine Food Technology). Wydawnictwa Naukowo-Techniczne, Warsaw (1971), Chap. 6.
4. H.A. BREMNER, Mechanically separated fish flesh from Australian species. A summary of results of storage trials. Fd. Technol. in Australia 30 (1978), p. 393.
5. H.A. BREMNER, G.M. LASLETT, and J. OLLEY, Taste panel assessment of textural properties of fish minces from Australian species. Fd. Technol 13 (1978), p. 307.
6. Z.E. SIKORSKI, Protein changes in muscle foods due to freezing and frozen storage. Bull. I.I.R. Comm. G1 and C2, Annexe 1977-1, Karlsruhe (1977).
7. V.D. TRAN, Effect of sodium pyruvate on the texture of frozen stored cod fillets. J. Fd. Scien. 40 (1975), p. 888.
8. R.M. LOVE, The effect of freezing of fish muscle. In "Recent advances in food Science", vol. 1, J. HAWTHORN and J.M. LEITCH

(eds.). Butterworth's, London (1962), p. 147.

9. T. TANAKA, Electron-microscope studies on toughness in frozen fish. FAO symposium on the significance of fundamental research in the utilization of fish. Paper No. W/P/III/10 (1964).
10. AOAC, (Official methods of analysis of the Association of Official Analytical Chemistry), 12th edition (1975).
11. A.A. LEACH, Notes on a modification of the Newman and Logan method for the determination of hydroxyproline. Biochemistry J. 74 (1960), p. 70.
12. R. TIMPL, R.W. GLANVILLE, H. NOWACK, H. WIEDEMANN, P. FRETZEK, and K. KÜHN, Hoppe-Seuler's Z. P. Phsiol. Chem. 356 (1975), p. 1783.
13. G. CHANDAKASAN, D.A. TORCHIA, and K. PIEZ, Preparation of intact monomeric collagen from rat tail tendon and skin and the structure of the nonhelical ends in solution. J. Biol. Chem. 251 No. 19 (1976).
14. T. KRIEG, C. LUDERSCHMIDT, L. WEBER, P.K. MULLER, and O. BRAUN-FALCO, Scleroderma fibroblasts: some aspects of in vitro assessment of collagen synthesis. Dermatological Res. 270 (1981), p. 263.
15. R. JACQUOT, Organic constituents of fish and other aquatic animal foods. In "Fish as Food", vol. 1, G. BORGSTROM (ed.). Academic Press, New York (1961), p. 145.
16. R.M. LOVE, The chemical biology of Fishes. Academic Press, London (1970).
17. J.M. MCBRIDE, R.A. MACLEOD, and D.R. IDLER, Seasonal variation in the collagen content of Pacific herring tissues. J. Fish. Res. Bd. Can. 17(6) (1960), p. 913.
18. J.I.M. IRONSIDE and R. LOVE, Studies on protein denaturation in frozen fish. Biological factors influencing the amounts of soluble and insoluble protein present in the muscle of the North Sea cod. J. Sci. Food Agric. 9 (1958), p. 597.
19. A. ASGHAR and R.C. HENRICKSON, Chemical, biochemical, functional and nutritional characteristics of collagen in food systems. Advances in Food Research 28. Academic Press, London (1982), p. 231.
20. T.J. SIMS and A. BAILEY, Connective tissue. In "Developments in meat science - 2", R. LAWRIE (ed.). Applied Science Publishers, London (1981), p. 28.

MODIFICATIONS DU COLLAGENE DU TISSU MUSCULAIRE DE POISSON CONGELE PENDANT LA CONSERVATION

RESUME : La présente étude traite des modifications que subit le collagène du tissu musculaire pendant la conservation de la chair de poisson congelée. Ces modifications peuvent, pense-t-on, être liées aux changements de texture qui se produisent pendant la conservation.

La solubilité (en solution saline et en solution acide) est une des propriétés étudiées. Les résultats indiquent qu'elle est fonction de l'espèce du poisson et des conditions de conservation. L'insolubilisation est importante chez le merlu tandis que la solubilité demeure inchangée chez la truite.

Au cours de la période de conservation, on observe une liaison progressive entre les chaînes α des fibres de collagène et les chaînes β et γ .

Cela donne une idée de l'agrégation moléculaire intervenant, pendant la conservation, dans le tissu musculaire congelé, ce qui, s'il est démontré qu'elle a un rapport avec les changements de texture (résultats à présenter dans une prochaine étude), pourrait expliquer certaines incohérences auxquelles on se heurte quand on essaie de rendre compte des changements de texture uniquement par des modifications des protéines myofibrillaires.

DISCUSSION

A. CORMIER (Canada) - Do you plan to determine melting points of insoluble collagen during your future work ?

A.J. BORDERÍAS - No. I plan to determine the melting point of acid soluble collagen.

L. HERBORG (Denmark) - Considering the tough texture which develops during storage how do you ensure uniform disintegration for mince samples stored for both short and long terms ?

A.J. BORDERÍAS - This toughening of texture does happen and it is necessary to break the sarcolemma that increases the toughness during frozen storage. In the case of the connective tissue there is no similar change to affect the solubilisation of collagen.

DOES FORMALDEHYDE FORM CROSS-LINKS BETWEEN MYOFIBRILLAR PROTEINS DURING FROZEN STORAGE OF FISH MUSCLE ?

H. REHBEIN

Institute of Biochemistry and Technology, Federal Research Centre of Fisheries, Palmaille 9, D-2000 Hamburg 50 (Fed. Rep. Germany)

1. INTRODUCTION

During frozen storage of fillets or minced flesh from fishes of the order Gadiformes formaldehyde (FA) is produced by enzymatic decomposition of trimethylamine oxide (TMAO) /1/; the concentration of FA can rise to 70 mmoles FA/kg wet weight depending on the storage temperature and fish species /2,3/ assuming an equimolar formation of FA and dimethylamine from TMAO. FA reacts with myofibrillar /4/ and sarcoplasmic /5/ fish muscle proteins decreasing their solubility in solutions of salt and buffer. Above all the myosin heavy chain (MHC) and tropomyosin are sensitive against FA as shown by electrophoretic studies (SDS-PAGE) /6,7/.

The mechanism of the reaction is unknown and no cross-linked muscle proteins could be detected /8/; oligomeres of the MHC are soluble in SDS-buffer, as demonstrated by cross-linking experiments using glutardialdehyde /9/ or transglutaminase /10/. The work described here was performed to examine the hypothesis of the existence of fish muscle protein aggregates formed by covalent bridges originating from FA /8/.

2. MATERIALS AND METHODS

Preparation and storage of saithe mince

Fillets from saithe (Pollachius virens; wet fish) were dissected in white and red muscle; the white muscle was homogenized and mixed with solutions of FA in distilled water (1 mL solution/100 g mince); the final concentrations of FA amounted to 0, 0.088, 0.88, 8.8 and 44 mmoles FA/kg wet weight. The minces were sealed in plastic bags, frozen in a stream of cold air (-40°C) and stored at -18°C .

The pH value of the minces was about 6.6 and did not change during frozen storage.

Fractionation and solubilization of saithe minces

From thawed minces the sarcoplasmic, the salt-soluble and the salt-insoluble fractions were prepared according to /11/. The salt-insoluble fraction, consisting of denatured myofibrillar proteins, was solubilized using SDS/borate-buffer (2% (w/v) SDS, 0.477 % (w/v) $\text{Na}_2\text{B}_4\text{O}_7 \times 10 \text{H}_2\text{O}$, 1 % (w/v) 2-mercaptoethanol (2-ME), pH 8.9); the samples were shaken in a water bath for 2 h at 100°C ; undissolved material was precipitated by centrifugation (30 min, 20°C , 38 000 g)

Isolation of crude myosin from carp muscle

The white muscle of carp (Cyprinus carpio) was taken from fish directly after slaughtering; crude myosin was isolated according to /12/.

Reaction of formaldehyde with crude myosin

To 8 mL of crude myosin solution (1.72 mg/mL 0.6 M NaCl, 15.6 mM Na_2HPO_4 , 3.5 mM NaH_2PO_4 , pH 7.5) were added 1 mL of FA solution (0.46 M FA in half-concentrated pH-buffer) and 1 mL of pH-buffer

(pH 6,7,8: 0.1 M Na-phosphate; pH 9: 0.1 M Na-borate); the mixtures were incubated for 4 h at 25 °C and centrifuged (20 min, 5 °C, 38 000 g). The supernatants were dialyzed overnight against 40 volumes of NaCl/phosphate-buffer at 0 °C.

The sediments were mixed with 5 mL of SDS/borate-buffer with/without 1 % (w/v) 2-ME, incubated in the refrigerator overnight, then stirred for 30 min at room temperature and heated within 15 min up to 100 °C followed by stirring at this temperature for 30 min. Undissolved material was precipitated by centrifugation (30 min, 20 °C, 38 000 g).

Analytical methods

FA was measured in perchloric acid extracts and in distillates of the minces as described in /13/. Steam distillation of acidified or alkalinized minces was performed according to /14/.

The conditions of SDS-PAGE (7.5 % gels) and of the estimation of protein and crude protein contents have been described recently /15/.

The texture of raw minces was determined with the Wolodkewitsch-Consistency-Tester /16/; the samples were packed into a case and pressed by a piston through a narrow circular slit. Cooked samples were tested using the Instron Universal Testing Machine Model 1140 equipped with the modified 4-bladed Kramer Shear Compression Cell /17/.

3. RESULTS AND DISCUSSION

Frozen storage of saithe minces treated with formaldehyde

Mince prepared from the white muscle of saithe had been supplied with maximal 44 mmoles FA/kg wet weight and stored at -18 °C for about 1 year. The effects of the different amounts of added FA on the texture and protein solubility of the minces are described in table I;

Table I

FA content, protein solubility and texture of saithe minces which had been stored for 13.5 months at -18 °C

	FA addition (mmoles FA/kg mince)			
	0	0.88	8.8	44
FA (mmoles/kg) extracted:				
with perchloric acid	0.11	0.28	0.44	7.35
from acidified mince	<1.00	2.18	10.2	47.3
from alkalinized mince	1.16	1.78	4.13	20.6
Sarcoplasmic protein (%) ^a	22.4	17.0	14.5	1.40
Salt-soluble protein (%)	10.7	7.03	7.29	3.72
Salt-insoluble protein (%)	52.3	61.3	64.9	81.9
Percentage of salt-insoluble protein solubilized by SDS/borate-buffer containing 1 % 2-ME (2 h, 100 °C)	91	104	104	106
Texture (N) ^c : raw mince	0.21 ± 12 %	0.32 ± 8.1 %	0.84 ± 10 %	>2.55 ^b ± 0.0 %
cooked mince	750 ± 0.0 %	733 ± 1.6 %	1143 ± 7.3 %	3483 ± 0.8 %

- a) percentage of crude protein of the mince
- b) no extrusion
- c) mean value and coefficient of variation from 4 (raw minces) or 3 (cooked minces) measurements

the following points shall be emphasized:

1. Most of the added FA could not be extracted with cold perchloric acid; by acid steam distillation FA was separated quantitatively.
2. Not only the myofibrillar proteins reacted with FA, but also the sarcoplasmic ones; similar results had been obtained with cod minces treated with FA /5/. Isoelectric focusing of sarcoplasmic proteins extracted from minces of various gadoid fishes showed that FA reacted mainly with basic proteins /18/. 3. The fraction of salt-soluble protein was very small even in those minces containing low amounts of FA. The denatured (salt-insoluble) protein could be solubilized completely using SDS/borate-buffer.

The SDS-PAGE of the salt-soluble protein fraction (Fig. 1A) resulted in patterns possessing the same number of bands in the case of the samples supplied with up to 8.8 mmoles FA/kg; the MHC became

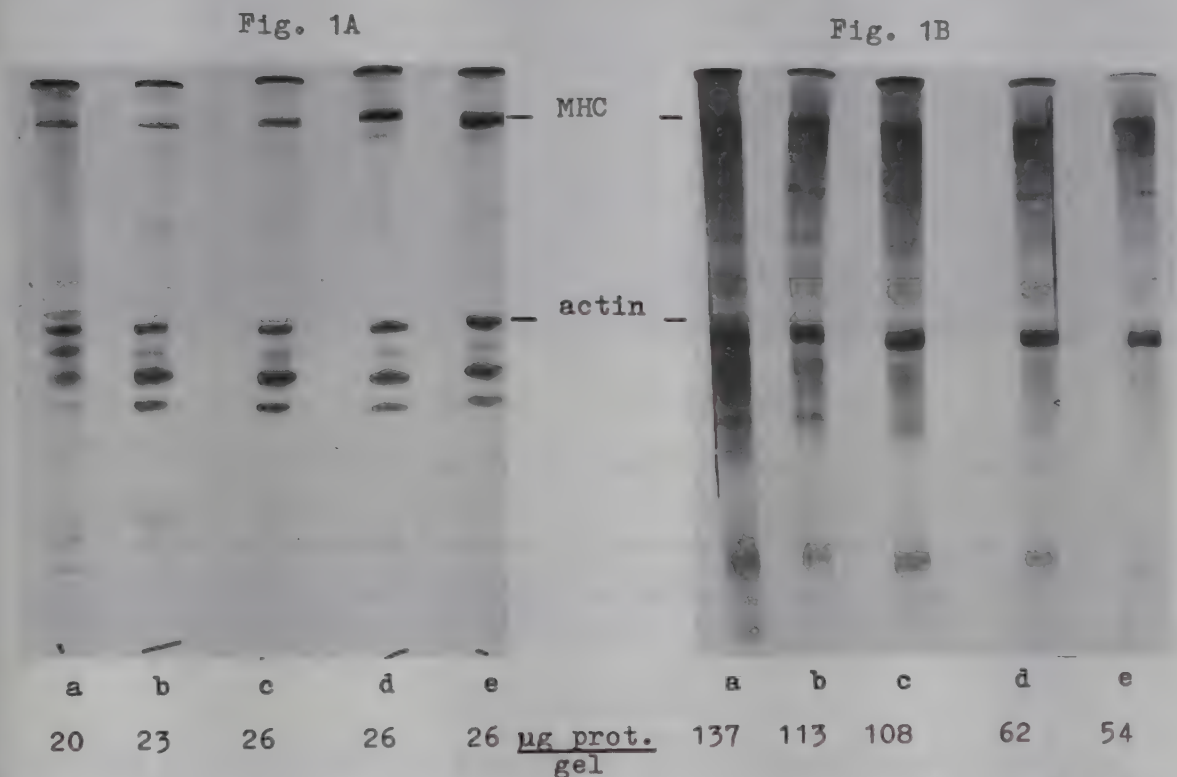


Fig. 1. - SDS electropherograms of the salt-soluble (Fig. 1A) and salt-insoluble (Fig. 1B) protein fractions from saithe minces treated with (a) 44, (b) 8.8, (c) 0.88, (d) 0.088 and (e) 0 mmoles FA/kg. The salt-soluble proteins were loaded with SDS by mixing 3 mL of protein solution with 1 mL of SDS/borate-buffer and incubation in boiling water for 10 min. The salt-insoluble fraction was solubilized as described in Materials & Methods.

weaker with increasing FA concentration. However the mince treated with 44 mmoles FA/kg gave an additional protein band (molecular weight: 50 k daltons) above actin; furthermore one low molecular weight band (33 k daltons) was weakened considerably. The new band may be produced by FA mediated cross-linking of low molecular weight myofibrillar proteins.

Salt-insoluble protein fraction (Fig. 1B): The gels exhibited an

intensive background staining resulting from the protein degradation at 100 °C /15/; the fundamental protein pattern was the same for all samples, MHC could be identified on every gel. A new protein band (50 k daltons) placed above actin appeared in the fraction from the mince treated with 44 mmoles FA/kg. Cross-linked MHC (e.g. dimers), identified as reaction products between glutardialdehyde and MHC /9/, could not be detected.

Reaction between formaldehyde and crude myosin from carp muscle

The capacity of FA as a protein cross-linker in frozen fish muscle may be limited by several reasons:

1. The distance between the protein molecules does not allow cross-linking by the short methylene bridges; under this aspect dimers between MHchains or between tropomyosin molecules should be formed preferentially because of the close proximity of these protein chains in coiled-coil structures. 2. FA can produce intramolecular cross-links in proteins /19/ which are not detectable in SDS-PAGE. 3. Mixed products formed by the reaction between proteins, FA and low molecular weight substances (e.g. free amino acids) may be formed /20/ without being noticed in SDS-PAGE or solubility studies. 4. The pH-values occurring in frozen fish muscle (6.2-7.0) are unfavourable for the reaction between FA and proteins /21/. 5. The FA concentration being available to the muscle proteins may be considerably lower than the measured amount of FA or dimethylamine, because fish muscle contains significant amounts of free amino acids, nucleotides and creatine which are able to trap FA /22/; in the fillet of cod (Gadus morhua) following concentrations (mmoles/kg wet weight) have been found: Σ free amino acids: 26.9, lysine: 2.37, histidine: 0.32, arginine: 0.32 /23/; creatine: 26.7 /24/; Σ ATP, ADP, AMP: 6.6 /25/. The total concentration of all these metabolites (63.2 mmoles/kg) is in the same range as the amount of lysine in cod muscle (98 mmoles/kg) /26/.

Table II

Precipitation of crude myosin by formaldehyde

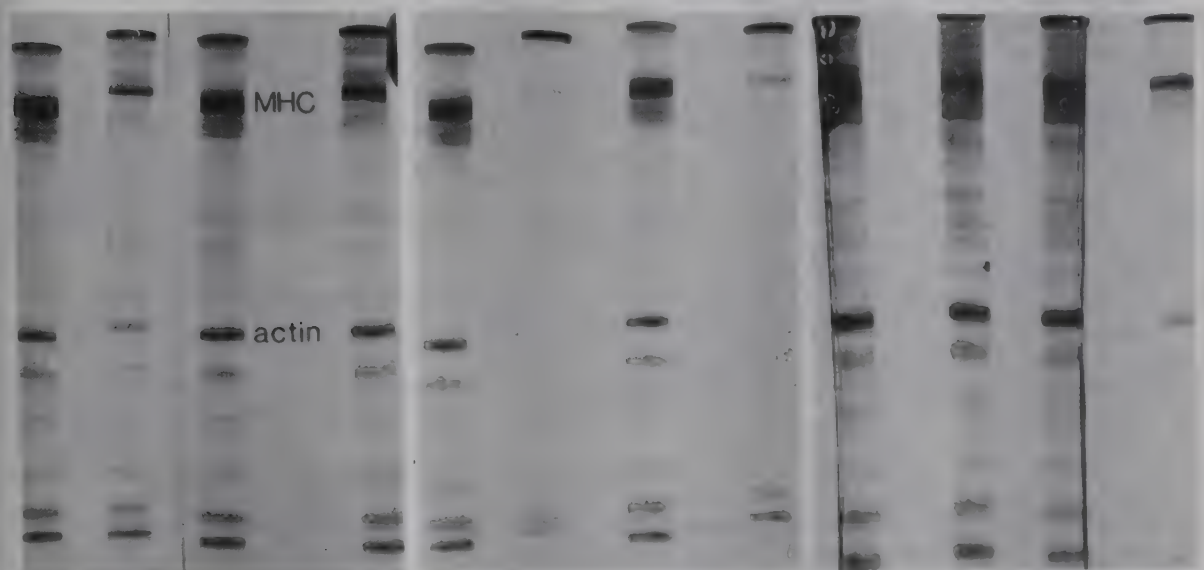
Test no.	pH of the assay	Presence of FA	Fraction of protein (% starting material)		
			Supernatant	Sediment solubilized $\oplus$ 2-ME	Sediment solubilized $\ominus$ 2-ME
1	6.64	+	86		5.1
2	6.65	+	91	10	
3	6.35	-	88	4.7	
4	7.05	+	47		25
5	7.10	+	44	43	
6	7.05	-	98	5.8	
7	7.60	+	20		8.0
8	7.61	+	24	57	
9	7.65	-	96	5.8	
10	8.80	+	25		12
11	8.76	+	17	58	
12	8.85	-	91	5.4	

The reaction between FA and myofibrillar proteins in a defined system was studied by incubating crude myosin from carp muscle with 46 mM FA at pH 6.4-8.9. Table II demonstrates the pronounced increase

of the reactivity of crude myosin towards FA when the pH rises from 6.6 to 7.6. Precipitated protein could be solubilized largely by SDS/borate-buffer, but the omission of 2-ME greatly reduced the solubilization power of the buffer.

Figure 2A-C shows that the crude myosin contained significant amounts of actin and regulator proteins; all proteins reacted with FA nearly to the same extent. New bands, indicating cross-linking of proteins by FA, did not appear. Obviously FA is able to denature myofibrillar proteins without the formation of covalent cross-links.

Fig. 2A-Supernatants Fig. 2B-Supernatants Fig. 2C-Sediments



Test no.:												
6	4	3		1	9	7	12	10	11	8	5	2
Formaldehyde:												
-	+	-		+	-	+	-	+	+	+	+	+
pH value:												
7.1	7.1	6.4		6.6	7.7	7.6	8.9	8.8	8.8	7.6	7.1	6.7

Fig. 2.- SDS-electropherograms of the supernatants and solubilized sediments from the reactions of crude myosin with FA.

4. ACKNOWLEDGEMENTS

I wish to thank Mrs. G. Kress for skilful technical assistance

REFERENCES

1. H. REHBEIN and W. SCHREIBER: TMAO-ase activity in tissues of fish species from the northeast atlantic. *Comp. Biochem. Physiol.* 79 B (3), (1984), pp. 447-452.
2. C.H. CASTELL, B. SMITH and W. NEAL: Production of dimethylamine in muscle of several species of gadoid fish during frozen storage, especially in relation to presence of dark muscle. *J. Fish. Res. Bd. Canada* 28 (1), (1971), pp. 1-5.
3. J. J. LICCIARDELLO, E.M. RAVESI, R.C. LUNDSTROM, K.A. WILHELM, F.F. CORREIA and M.G. ALLSUP: Frozen storage stability of red hake fillet blocks. In: *Advances in the refrigerated treatment of fish. I.I.F.-I.I.R.-Commissions C2, D1, D2, D3- Boston (U.S.A.)-1981-4*, pp. 377-382.

4. Z. SIKORSKI, J. OLLEY and S. KOSTUCH: Protein changes in frozen fish. *C.R.C. Crit. Rev. Fd. Sci. Nutri.* 8 (1), (1976), pp. 97-129.
5. R.G. POULTER and R.A. LAWRIE: Studies on fish muscle protein: nutritional consequences of adding low concentrations of formaldehyde and/or linoleic acid to cod muscle. *Lebensm. Wiss. u. Technol.* 12 (1), (1979), pp. 47-51.
6. E.A. CHILDS: Interaction of formaldehyde with fish muscle in vitro. *J. Food Sci.* 38, (1973), pp. 1009-1011.
7. M. OHNISHI and G.W. RODGER: Effect of formaldehyde addition at different ionic strengths on the salt-soluble proteins of fish muscle. In: *Advances in Fish Science and Technology*, ed. J.J. Connell. Fishing News (Books) Ltd., (1980), pp. 459-467.
8. A.D. MATTHEWS, G.R. PARK and E.M. ANDERSON: Evidence for the formation of covalent cross-linked myosin in frozen stored cod minces. In: *Advances in Fish Sciences and Technology*, ed. J.J. Connell. Fishing News (Books) Ltd., (1980), pp. 438-444.
9. E. REISLER, M. BURKE, R. JOSEPHS and W.F. HARRINGTON: Crosslinking of myosin and myosin filaments. *J. Mechanochem. Cell Motility* 2, (1973), pp. 163-179.
10. L. KURTH and P.J. ROGERS: Transglutaminase catalyzed cross-linking of myosin to soya protein, casein and gluten. *J. Food Sci.* 49, (1984), pp. 573-589.
11. K. HASHIMOTO, S. WATABE, M. KONO and K. SHIRO: Muscle protein composition of sardine and mackerel. *Bull. Jap. Soc. Sci. Fish.* 45 (11), (1979), pp. 1435-1441.
12. T. TSUCHIYA and J.J. MATSUMOTO: Isolation, purification and structure of carp myosin, HMM and LMM. *Bull. Jap. Soc. Sci. Fish.* 41 (12), (1975), pp. 1319-1326.
13. T. NASH: The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem. J.* 55, (1953), pp. 416-421.
14. N. ANTONACOPOULOS: Bestimmung von Hexamethylentetramin (Formaldehyd). In: *Fische und Fischerzeugnisse*, W. Ludorff and V. Meyer. Paul Parey Publishers, Berlin, (1973), pp. 231-232.
15. H. REHBEIN and H. KARL: Solubilization of fish muscle proteins with buffers containing sodium dodecyl sulfate. *Z. Lebensm. Unters. Forsch.* (1985), in the press.
16. T. GRUENEWALD: Ein Festigkeitspruefgeraet fuer Lebensmittel nach N. Wolodkewitsch. *Z. Lebensm. Unters. Forsch.* 105, (1957), pp. 1-12.
17. E. BILINSKI, V.C. LAW and R.E.E. JONAS: Objective measurement and control of the firmness of canned herring. Fisheries and Marine Service, Canada, Technical Report No. 727, (1977).
18. H. REHBEIN: Fish species identification by isoelectric focusing of sarcoplasmatic proteins: changes of the species specific protein patterns in dependence of the fishing ground and the conditions of frozen storage of the samples. In: *Electrophoresis '84*, ed. V. Neuhoff, Verlag Chemie, Weinheim, (1984), pp. 471-474.
19. J.S. MYERS and J.K. HARDMAN: Formaldehyde-induced cross-linkages in the alpha-subunit of the Escherichia coli tryptophan synthetase. *J. Biol. Chem.* 246 (12), (1971), pp. 3863-3869.
20. J.R. WARREN, L. SPERO and J.F. METZGER: The pH dependence of enterotoxin polymerization by formaldehyde. *Biochim. Biophys. Acta* 365, (1974), pp. 434-438.
21. F. GALEMBECK, D.S. RYAN, J.R. WHITAKER and R.E. FEENEY: Reaction of proteins in the presence and absence of sodium borohydride. *J. Agric. Food Chem.* 25 (2), (1977), pp. 238-245.
22. Y. KITAMOTO and H. MAEDA: Reevaluation of the reaction of formaldehyde at low concentration with amino acids. *J. Biochem.* 87, (1980), pp. 1519-1530.
23. I.M. MACKIE and A.H. RITCHIE: Free amino acids of fish flesh. *Proc. IV. Int. Congress Food Sci. and Technol.*, (1974), Vol. I., pp. 29-38.
24. J.M. SHEWAN: The chemistry and metabolism of the nitrogenous extractives in fish. In: *The biochemistry of fish*, ed. R.T. Williams, Cambridge at the University Press, (1951), pp. 28-47.

25. N.R. JONES and J. MURRAY: Nucleotides in the skeletal muscle of codling (Gadus callarias). Biochem. J. 66, (1957), 5p-6p.
26. V.D. SIDWELL: Chemical and nutritional composition of finfishes, whales, crustaceans, mollusks, and their products. NOAA Technical Memorandum NMFS F/SEC-11, (1981), p. 51 and p. 140.
-

LA FORMALDEHYDE FORME-T-ELLE DES LIAISONS CROISEES ENTRE LES PROTEINES MYOFIBRILLAIRES AU COURS DE L'ENTREPOSAGE DE MUSCLE DE POISSON CONGELE ?

RESUME: Des muscles lisses de lieu noir ont été hachés et entreposés à l'état congelé pendant un an à -18°C , après avoir ajouté de la formaldehyde (FA) à raison de 0-44 mmol/kg de produit humide. L'analyse électrophorétique (SDS-PAGE) des protéines myofibrillaires a démontré que les chaînes lourdes de la myosine n'ont pas été liées de façon covalente par action de la FA. Une forte augmentation de la concentration de la FA provoquait l'apparition d'une nouvelle bande (masse moléculaire de 50 K daltons).

De la myosine brute a été isolée des muscles lisses de carpe et a réagi avec FA à un pH de 6.6-8.8. La quantité de myosine ainsi précipitée augmentait parallèlement avec le pH, les précipités se dissolvaient dans une solution de SDS (2 %) et de 2-mercapto-éthanol (1 %) dans un tampon à un pH de 8.9 - on observait aucun signe de protéines oligomérisées ou polymérisées lors de l'électrophorèse de ces solutions.

DISCUSSION

T. BØRRESEN (Denmark) - 1) First, a comment on the reactivity of formaldehyde (FA) with proteins. The presentation shows that FA reacts with all protein fractions, but this is not surprising, because of FA's high reactivity.

2) The chemical bond which FA forms when reacting with protein is unstable (Schiff's base). Have you tried to stabilise the bonds formed chemically, before analysis ?

H. REHBEIN - This has been done, but only small amounts of methyl lysine were formed. The cross-linking of two lysines by formaldehyde are considered to be stable.

SECTION III

ENTREPOSAGE DES POISSONS ET PRODUITS
DE LA MER CONGELÉS

STORAGE OF FROZEN FISH AND SHELLFISH

EFFECT OF RAW MATERIAL QUALITY ON THE SHELF-LIFE OF FROZEN SQUID (LOLIGO DUVAUCELLI) MANTLES

JOSE JOSEPH, P.A. PERIGREEN & M.R. NAIR
Central Institute of Fisheries Technology,
Cochin-682029, India.

INTRODUCTION

Squid is one of the important marine fishery resources of India. The landing of cephalopods increased from 7889 tonnes in 1975 to 16,000 tonnes in 1982 and about half of the quantity is contributed by squid. The export of frozen squid was 46 tonnes in 1975 which increased to 2217 tonnes during 1983 (1). Since it is considered unconventional in many parts of India, a negligible portion of it is consumed locally. This shows that a significant quantity of squid is wasted every year. This is due to the rapid spoilage and the difficulties in handling fresh squid. The squid is mainly caught in trawl nets in India. Several workers in India and abroad have studied various aspects of handling, processing and frozen storage of squid (2,3,4,5,6). The effect of season on raw material quality was studied by Takahashi (7) and found that the squid caught in summer deteriorated faster than those captured in autumn. Moral, *et al.* (5) found a shelf-life of more than one year for squid, though it is highly susceptible to desiccation during frozen storage.

The present investigation was carried out to study the various problems associated with handling and processing of squid and the effect of raw material quality on the shelf-life of squid during frozen storage.

MATERIALS AND METHODS

Extremely fresh squids (Loligo duvaucelii) having average weight 175 g were collected on board CIFT research vessel from the trawl catch. They were washed well and kept under ice in insulated box. The squids were brought to the laboratory immediately after landing and divided into three batches and the following operations were conducted within 8 hours after catch.

- B-I Dressed mantles were washed, frozen immediately and stored at $-20 \pm 1^{\circ}\text{C}$.
- B-II Dressed mantles were kept in ice for one day, frozen and stored at $-20 \pm 1^{\circ}\text{C}$.
- B-III The whole squids were kept in ice and after one day part of it was taken out and the dressed mantles were frozen and stored at $-20 \pm 1^{\circ}\text{C}$.

In all the cases freezing was done in a contact plate freezer after packing the samples (450 g) in waxed cartons lined inside with 100 gauge polythene sheet. The remaining samples of B-III were used for iced storage studies.

The frozen samples were thawed by keeping them in air tight polythene bags in running water. The weight of the samples were taken before and after thawing and expressed as percentage based on the

original weight. The thawed material was used for analysis. The cook drip was determined by weighing the sample before and after cooking for 10 minutes in boiling 2% brine and expressed as percentage on the basis of weight of the sample before cooking.

The samples were analysed periodically for moisture, non-protein nitrogen and total nitrogen by the methods of A.O.A.C. (8), extractable protein by the method of Dyer, et al. (9), alpha amino nitrogen from the trichloro acetic acid extract by the method of Pope & Stevens (10) and trimethylamine nitrogen (TMAN) by the micro-diffusion method of Conway (11). For organoleptic studies the samples were cooked in 2% boiling brine for 10 minutes and presented to a trained taste panel of 10 members and asked to score for overall acceptability in a scale ranging from 1 to 9, 1 for extremely spoiled, 4 dislike slightly, 5 neither like nor dislike and 9 like extremely. The panel was asked to make comments on any important observation.

RESULTS AND DISCUSSION

The visual changes during iced storage of squid are given in Table I. The whole squid developed pink discolouration in the mantles

Table I. Changes during iced storage of squid

Days	Whole squid	Mantles
1	No noticeable change in the mantles	Good white colour
2	Pink discolouration on the mantles	White colour, mantles became transparent
3	Pink discolouration, development of pale yellow colour near the belly portion, slight off odour	Mantles became thin and easily breaking
4	Significant decay near the belly portion, yellow discolouration	Mantles became very thin, no off odour

by keeping two days in ice. The intensity of the pink colour was reduced on the third day, but the belly portion of the mantles turned pale yellow. This was accompanied by off odour. The intensity of the yellow discolouration increased and spread on further iced storage with an increasing stale odour. The cooked material had a dull brown appearance and the characteristic sweet taste was significantly decreased. By dressing the squid immediately after capture, the pink and yellow discolouration was avoided, but it could not be kept in direct contact with ice for longer periods because of the rapid leaching of soluble proteins and non-protein components (3) as a result of which the characteristic sweet taste of squid was lost within two days in ice.

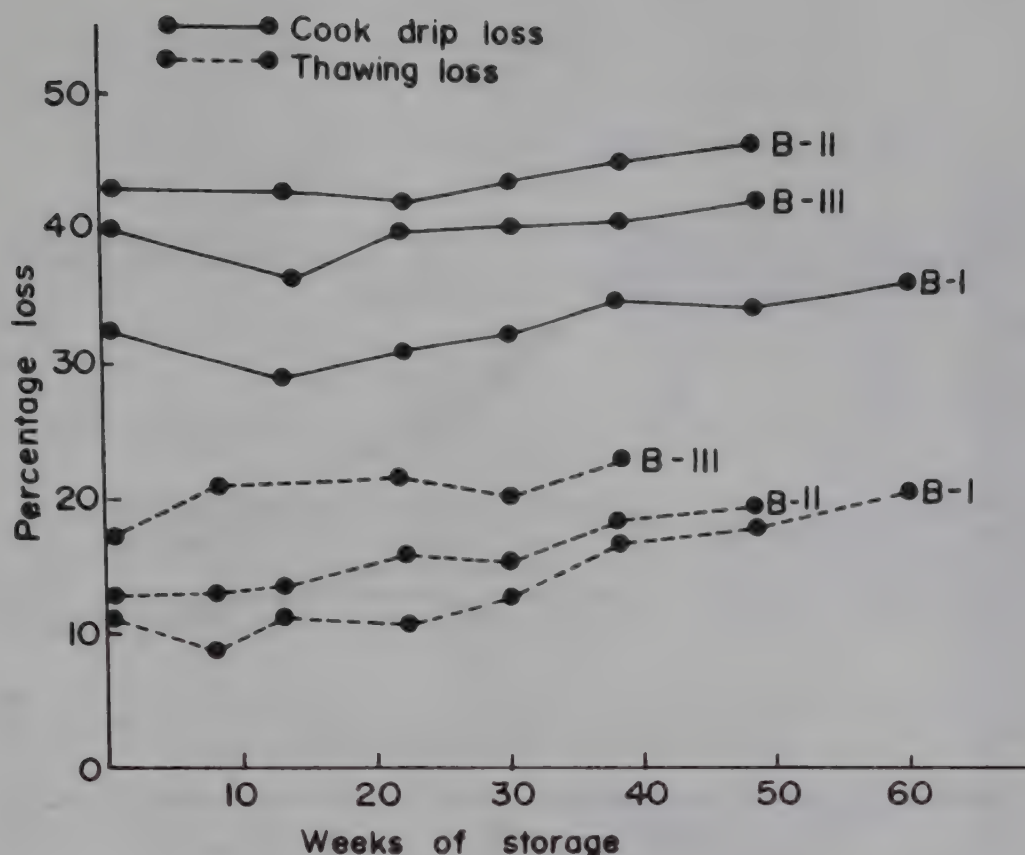


Fig.1. Weight loss on thawing and cook drip loss of frozen squid

The percentage weight loss on thawing and cook drip loss are shown in Fig.1. B-III showed the maximum amount of thaw drip and it was minimum in B-I. The thaw drip remained more or less constant in B-I for 30 weeks and afterwards showed an increase. Cook drip was maximum in B-II. An increase in cook drip loss was shown in all the samples by 48 weeks storage. The change in moisture content of frozen samples are shown in Fig.2.

The changes in protein extractability are shown in Fig.3. All the samples showed high values initially and the decrease in extractability was only gradual. In sample B-I a substantial decrease was noticed by 48 weeks storage.

Table II shows the changes in TMAN during frozen storage. The TMAN increase of B-II and B-III was negligible while B-I showed an increase. It might be due to the formation of dimethylamine (DMA). The conversion of trimethylamine oxide (TMAO) to DMA and formaldehyde (FA) is catalysed by an enzyme system present in the sarcoplasmic fraction of fish, squid and clams (12). Tokunaga (13) observed that fish stored several days before freezing accumulated less FA during frozen storage than corresponding samples when fresh.

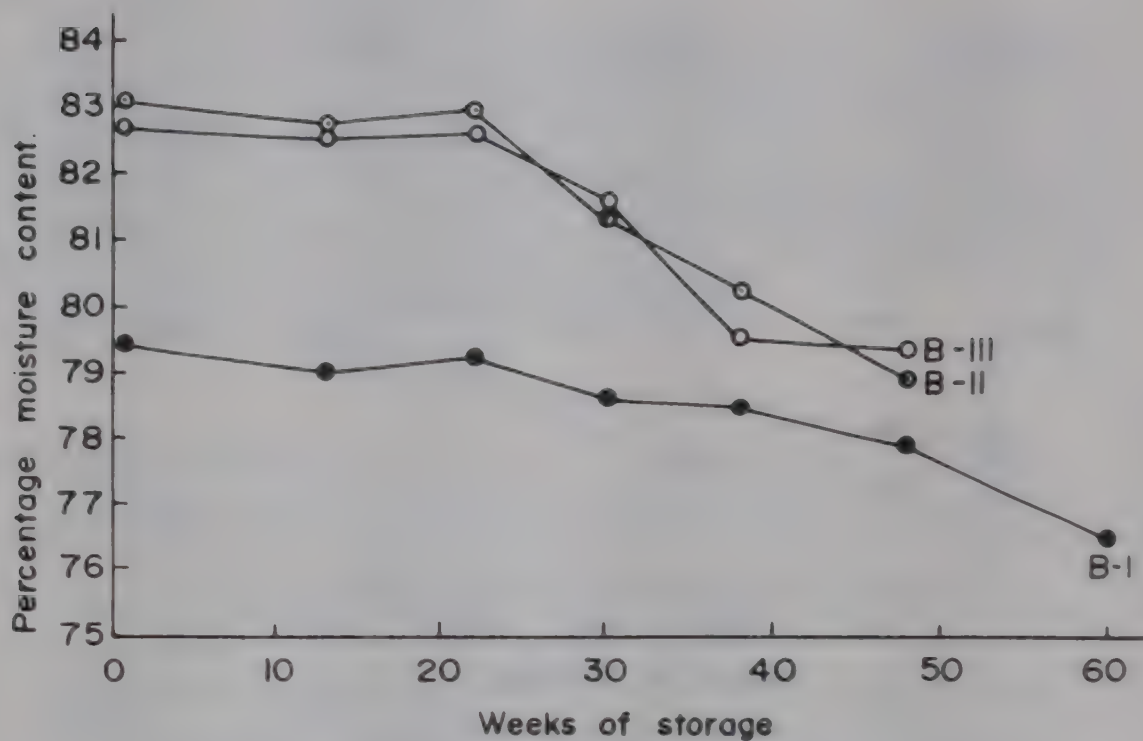


Fig.2. Changes in moisture content of squid during frozen storage

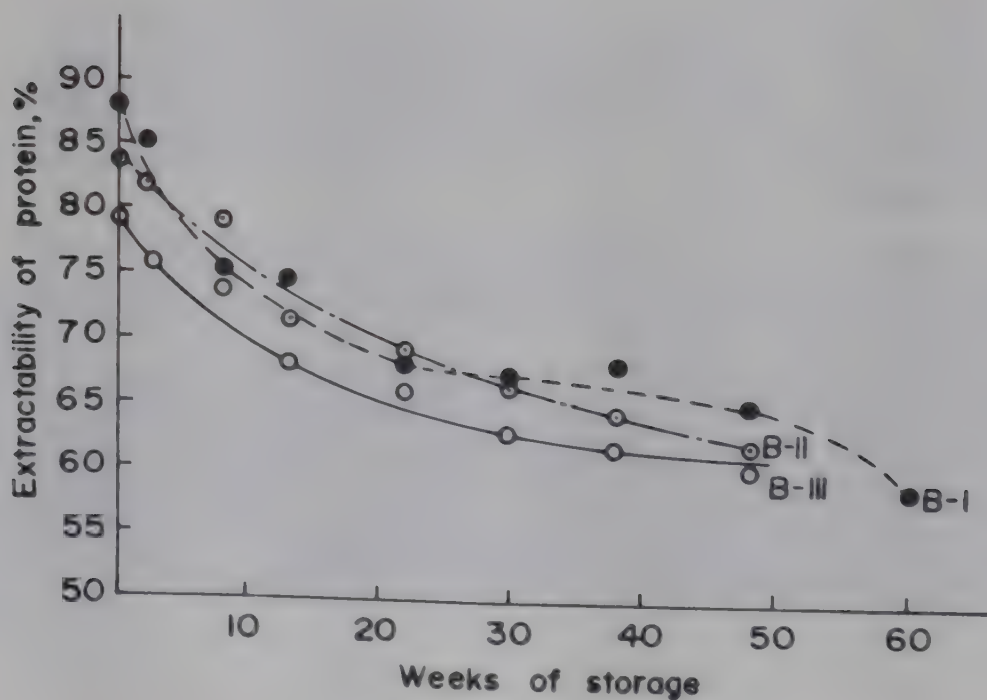


Fig.3. Changes in extractability of protein in frozen squid

Table II. Changes in TMAN of squid during frozen storage

No. of weeks	B-I	B-II	B-III
0	Negligible	1.4	2.6
2	"	1.4	2.4
8	"	1.4	2.4
22	2.4	1.8	2.8
30	2.6	1.6	2.8

The amounts of alpha amino nitrogen in thawed samples are shown in Table III. B-I had a high value of alpha amino nitrogen and lowest in B-II. The organoleptic evaluation of taste was related to amount of alpha amino nitrogen present. Simidu & Taneka (14) attributed the sweet taste to the presence of high amounts of alpha amino acids.

Table III. Changes in alpha amino nitrogen contents of squid during frozen storage

No. of weeks	Alpha-amino nitrogen (mg/100 g)		
	B-I	B-II	B-III
0	238	81	113
2	231	72	105
8	213	68	98
13	190	58	94
22	171	50	76
30	173	53	-
38	162	51	67
48	144		
60	122		

The organoleptic ratings of the samples during frozen storage are shown in Fig.4. There was significant difference in sensory characteristics between B-I and other two samples. B-I showed a gradual decrease in the rating and was in acceptable condition upto 60 weeks storage. Ice crystal formation on the surface and slight desiccation was observed by 22 weeks. This did not greatly affect the organoleptic rating. Moral, *et al.* (5) observed that squid was very sensitive to freezer burn. The amount of ice crystals and intensity of desiccation increased on further storage. B-II and B-III were in satisfactory condition for about 38 weeks and B-III were found to be better though the colour was duller than B-II. B-III retained some of characteristics flavour and texture was also good.

It is difficult to assess the frozen storage life by following the chemical changes. No correlation was found between the texture and the extractability of frozen squid. Even when the texture which was reflected in the organoleptic rating was low the extractability was somewhat high. It was found that the raw material quality and the shelf-life on frozen storage were significantly affected by keeping dressed squid mantles or whole squid in direct contact with melting ice.

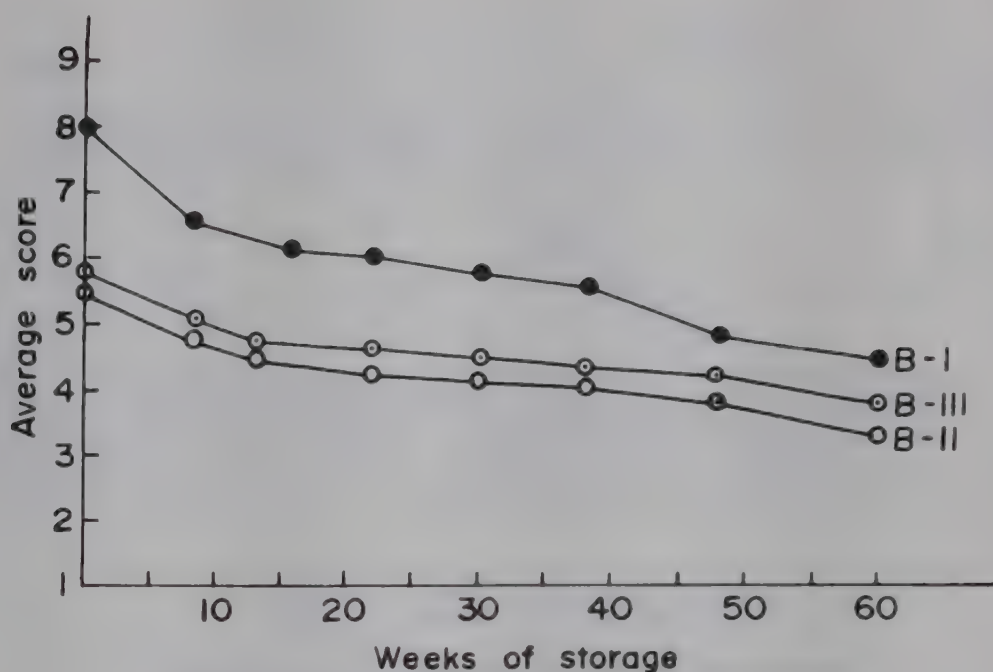


Fig.4. Organoleptic scores of squid during frozen storage

REFERENCES

1. Anon: Indian Marine Products Exports - 1983-84. The Marine Products Export Development Authority, Cochin, India.
2. Jose Joseph, P.R.G.Varma and R.Venketaraman: Iced and frozen storage of squid (*Loligo* sp.). Fish. Technol., Vol.12 (1977) pp.13-20.
3. M.R.Raghunath: Soluble nitrogen losses in squids (*Loligo duvaucelii*) during storage in slush ice. J. Food Sci. Technol., Vol. 21 (1984) pp.50-52
4. J.R.Botta, A.P.Downey, J.T.Lauder and P.B.Noonan: Preservation of raw whole short-finned squid (*Illex illecebrosus*) during the period from catching to processing. Fisheries and Marine Service Technical Report No.855, Dept. of Fisheries and Oceans, St. John's Newfoundland A1 C5 X1 (1979).
5. A.Moral, M.Tajeda and A.J.Borderias: Frozen storage behaviour of squid (*Loligo vulgaris*). International Journal of Refrigeration, Vol.6 (1983) pp.54-57.
6. S.J.Thrower: Catching and handling squid. Australian Fisheries, Vol.37(9) (1978) pp.20-25.
7. Toyo-O Takahashi: Squid meat and its processing. Fish as Food, Vol.4 (1965) pp.339-354, G.Borgstrom (Ed), Academic Press, Inc., New York.
8. A.O.A.C.: Official Methods of Analysis, 12th Edn., Association of Official Analytical Chemists, Washington (1975).
9. E.J.Dyer, M.V.French and J.H.Snow: Proteins in fish muscle. 1. Extraction of protein fractions in fresh fish. J. Fish. Res. Bd Can., Vol.7 (1950) pp.585-593.
10. C.G.Pope and M.F.Stevens: The determination of amino nitrogen using a copper method. Biochem. J., Vol.33 (1939) p.1070

11. E.J.Conway: Microdiffusion analysis and volumetric error. Crossby, Lockwood and Sons, London (1947).
12. Zdzislaw Sikorski, June Olley and Sylvia Kostuch: Protein changes in frozen fish. Critical Reviews of Food Science and Nutrition, Vol.8 (1976) pp.97-129
13. T.Tokunaga: Studies on the development of dimethylamine and formaldehyde in Alaska Pollack muscle during frozen storage. I. Torry Research Station, Translation No.743, Rep. Hokkaido Reg. Fish. Res. Inst., Vol.29 (1964) p.108.
14. W.Simidu and M.Takeda: Studies on muscle of aquatic animals. XII. Distribution of extractive nitrogen in muscles of squids. Bull. Jap. Soc. Sci. Fish., Vol.18 (1952) pp.233-236.

INFLUENCE DE LA QUALITE DE LA MATIERE PREMIERE SUR LA CONSERVATION DE MANTEAUX DE CALMAR (Loligo duvauceli) congelé.

RESUME : On a étudié l'influence de la qualité de la matière première sur la conservation de manteaux de calmar (Loligo duvauceli) CONGELE.
 - 20 ± 1 °C. L'ensemble du calmar présentait un rosissement du manteau après 2 jours dans la glace concassée, la partie intérieure du manteau devenait jaune pâle avec une légère odeur anormale qui augmentait avec l'entreposage. En préparant les calmars immédiatement après capture et en entreposant les manteaux dans de la glace on pouvait éviter le rosissement et le jaunissement, mais les manteaux ne pouvaient être maintenus dans de la glace plus de 2 jours par suite de la lixiviation rapide des composants azotés provoquant une perte de saveur caractéristique. Les manteaux provenant de calmars glacés à bord et congelés moins de 8 h après la pêche peuvent être conservés 60 semaines à $- 20 \pm 1$ °C, mais les calmars congelés provenant de manteaux glacés un jour après la pêche ou préparés avec du calmar entier glacé un jour après la pêche étaient inacceptables au bout de 38 semaines d'après les essais chimiques et organoleptiques. Les pertes dues à la décongélation et à la cuisson étaient minimales pour les manteaux congelés moins de 8 h après la pêche en comparaison des deux autres échantillons. La diminution de l'extractibilité des protéines était progressive et était importante (60 % environ) même après 48 semaines d'entreposage. On a observé une corrélation significative entre la teneur en α -azote aminé et la saveur caractéristique du calmar au cours de l'entreposage à l'état congelé.

DISCUSSION

Z. KARNICKI (FAO) - What is the reason for you not recommending direct icing ? This is done in other countries and it requires to be clarified, particularly since there is an attempt made by Codex Alimentarius to elaborate the International Code of Recommended Practice for Cephalopods.

K. GOPAKUMAR - The squid caught in India is Loligo duvancelli and studies have shown that if it is kept in contact with ice for long periods it develops a pink or yellow discoloration. We, therefore, recommend immediate icing followed by freezing within 24 hours after capture and if the ice is confined to the mantles rather than the whole squid this prevents pink discoloration.

R.M. GORDON (Guyana) - Did you consider any other methods of preserving the squid ?

K. GOPAKUMAR - Both sun and artificial drying have been used to produce squid rings and split-open and flattened dried products.

STUDIES ON THE FROZEN STORAGE OF FISH.

D.S. REID, N.F. DOONG, A. FOIN AND M. SNIDER
Department of Food Science and Technology
University of California,
Davis, California 95616, USA.

INTRODUCTION

The quality of fish maintained in frozen storage deteriorates steadily. This loss in quality is a significant factor in the utilization of fish. In order to minimize quality loss, very low temperature storage can be used. Such storage is costly. There is therefore always a tendency toward storing frozen fish at the highest practicable storage temperature to optimise the cost/benefit relationship. We do not, as yet, have a complete understanding of the processes of deterioration of fish during frozen storage. Changes are known to take place in the chemical/ biochemical composition of the fish. These are species dependent. Changes also take place in the structure of the tissue, as a consequence of freezing, and also continuously during frozen storage. There is little information available as to correlations which may or may not exist between structural change, and chemical/ biochemical change.

In this study, we are following the structural changes in frozen, stored fish using special microscopic procedures. In order to visualize the frozen tissues with a minimum of distortion, we employ the technique of isothermal freeze fixation, (IFF) in which a fixative, thermodynamically equilibrated with ice at the temperature of frozen storage, is used to chemically cross-link the non-ice matrix. In this way we can observe the structure of the frozen tissue, as the fixed tissue has cavities which correspond to the size and location of the ice crystals that were present. The tissues, appropriately fixed, can be later prepared for microscopic examination using optical microscopic techniques (LM), or electron microscope methods such as scanning electron microscopy (SEM) or transmission electron microscopy (TEM). Each of these techniques has its particular set of advantages and disadvantages. In order to study ice crystal size and distribution in the frozen tissues, LM is an appropriate tool. The sizes of the ice crystals are such that the range of magnifications available to LM is quite appropriate. TEM is not suited to the study of the ice cavities, as such, but is appropriate for the study of the state of the unfrozen matrix material between ice crystals. SEM gives good indications of the frozen structures, and has available to it a wide range of magnifications.

We report here mainly optical microscope data on the frozen structures of fish tissues, which have been frozen either slowly or rapidly, and then have been placed in frozen storage at one of three temperatures. In addition we report data on the changing biochemical status of these fish, obtained by measuring such parameters as myofibrillar protein solubility, protein ATPase activity, water holding capacity, tissue homogenate pH, and viscosity. In the period of the study being here reported, not all of these indicators exhibited significant change, but they would be expected to change over the full duration of the study, which is still in progress.

EXPERIMENTAL METHODS

The fish used in these studies are seabastes species, caught in the Pacific Ocean off the San Francisco - Fort Bragg coastal region in California and processed in our pilot plant as soon as practicable after catching. Fish were stored on ice prior to processing. The fish were filleted on arrival at Davis, the fillets washed, obvious red muscle was removed, and approximately 0.5 to 1 lb of fish placed in a deep tray and wrapped to prevent moisture loss. The sample was then frozen in the deep tray, using a blast freezer operating at -69°C . The temperature change in selected samples was monitored by means of a thermocouple located close to the thermal center. The fish was removed after an appropriate amount of heat had been extracted. The frozen fish was sealed in a freezer bag to protect it from moisture loss during frozen storage, and placed in the appropriate storage environment. Slow frozen fish was packaged in tray as before, and placed directly in the storage environment.

I.I.F.- I.I.R. - Commissions C2, D3.- Aberdeen (United Kingdom) - 1985-4

Isothermal freeze fixation

Isothermal freeze fixatives are prepared to have a melting point only slightly above the temperature of fixation. They contain 0.1M cacodylate as buffer to maintain pH at 6.3. The fixative is glutaraldehyde at about 3% concentration. Sufficient dimethyl sulfoxide is added to adjust the freezing point of the fixative solution to about 1 - 2 degrees above the fixation temperature, in order that the fixative at its working temperature has a small amount of ice present. For -20°C the DMSO concentration is approximately 35%. Washing solutions, in which the glutaraldehyde is replaced by glycerol can also be used, if fixative has to be removed prior to thawing. Table 1 lists some typical formulations. In order to fix frozen tissues, cores are removed from the frozen material, and placed, still frozen, in the appropriate fixative. The containers with cores undergoing fixation are stored in freezer baths at the appropriate temperatures until fixation is complete, and then the cores, now isothermally freeze fixed, are removed and prepared for examination by the standard LM, SEM, and TEM procedures for fixed materials.

Myofibrillar protein solubility

Homogenized light muscle was measured for myofibrillar protein solubility by the method of Hashimoto et al (1979). All operations were performed at $3-4^{\circ}\text{C}$. A 20g sample was homogenized in 200 ml of 0.05M phosphate buffer, pH 7.5. The mixture was centrifuged at 5000G for 15 min. After removing the liquid fraction, the extraction was repeated on the residue. The residual material from the second extraction was homogenized with 10 volumes of $I = 0.5$ KCl/phosphate buffer, pH 7.5 and centrifuged. This step, too, was repeated. The supernatants of the $I = 0.5$ buffer treatment make up the myofibrillar protein fraction. The protein content is assayed by the biuret method (Gornall et al (1949)).

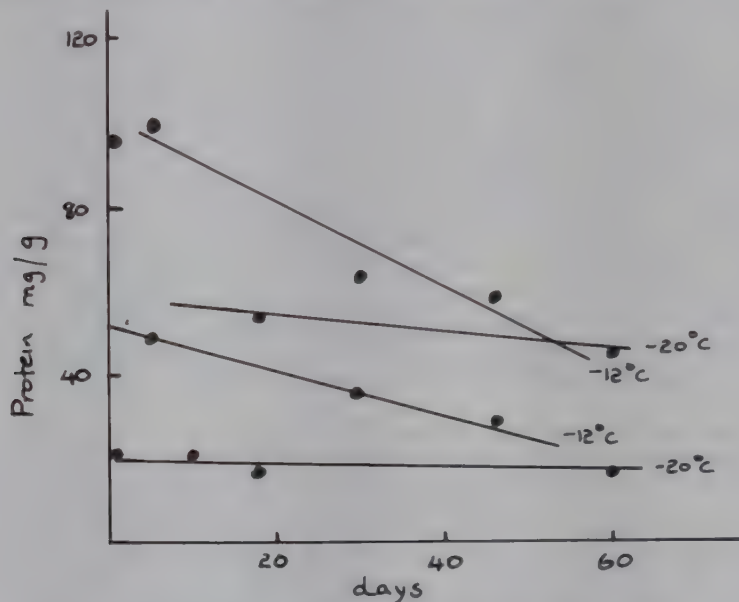


Figure 1: Soluble protein fractions extracted from light muscle after frozen storage

ATPase activity

ATPase activity was determined on a myofibrillar fraction, which was assayed for enzyme activity by adding ATP substrate. Ground fish was homogenized with 3 volumes of a 0.1MKCl/40mM borate buffer (pH7). Care was taken to ensure adequate homogenization. The homogenate was diluted into 17 volumes of 0.1M KCl, then centrifuged at 1300G for 10 minutes. The pellet was purified by repeated cycles of resuspension in 0.1M KCl, and centrifugation. The final pellet was resuspended in 0.1M KCl, and the protein content determined by biuret. ATPase assays were performed in duplicate at 25°C . 5ml of protein solution, adjusted to a concentration of 0.25mg/ml was used. The reaction mixture also contained 0.1M KCl, 20mM tris maleate (pH 7), 1mM ATP. The mixture also contained either 5mM CaCl_2 or 1mM MgCl_2 . The reaction was initiated by the addition of the ATP to the other components, previously mixed. After 5 minutes, the reaction was stopped by adding 1ml of cold 15% trichloroacetic acid, which precipitates the protein. After immediate chilling, and centrifugation to remove the precipitate, the supernatant was assayed for liberated phosphate. A protein free blank was performed.

Water holding capacity

The centrifugal method of Eide et al was employed. Two gram of homogenized flesh were placed in a tared stoppered holder and weighed. The sample holder, a tube with a mesh bottom was then placed in a centrifuge tube half filled with glass beads. The assembly was spun for 5 min at 1500g. The sample weight loss was determined immediately after centrifugation.

Homogenate pH

The pH of the flesh was determined after homogenising in 4 volumes of water.

Homogenate viscosity

The viscosity of the fish muscle was determined after homogenising in 4 volumes of 3%NaCl. A modification of the method of Groninger et al (1983) was used.

RESULTS

The amount of ice in frozen fish is a function of the storage temperature. As the photomicrographs (figures 3-5) clearly show, there is much less ice at the higher temperatures. During frozen storage, whilst the amount of ice does not change, the size of the individual ice crystals increases. This can be seen in the optical micrographs which relate to frozen tissue stored at -12°C for different periods. Note that, should the tissue be allowed to thaw prior to fixation, much of the information on the size and location of the ice crystals is lost. This is clearly shown by Lampilla, Mohr and Reid.(1985). Scanning electron micrographs of tissue isothermally freeze fixed at -5° and -20°C clearly show cavities due to the ice in the frozen tissue.

In addition to studying structural changes taking place in the frozen tissues, we are collecting data on the changing biochemical status of the fish. As the figures show, the change is more rapid at -12°C , than at -20°C . We are attempting to correlate the change in biochemical status with the structural changes taking place in the frozen tissues. As storage time increases, the structural damage increases. This is clear from the sequence of micrographs for -12°C . The biochemical change also increases. At present no obvious correlation between structure and status has emerged. However, we are still processing data for all storage temperatures relating to samples frozen under different conditions, which appear to have very different frozen structural features. Any correlation between structure and status would be expected to show up here. Also, we are still in the process of quantifying the oxidative changes in the fish lipid fractions. Here, particularly, one might expect to see a correlation with frozen structure, as both the rejection of oxygen from ice, and the diffusion of oxygen around ice crystals might be expected to depend on the exact frozen structure. The oxidized lipids might then be expected to affect protein solubility.

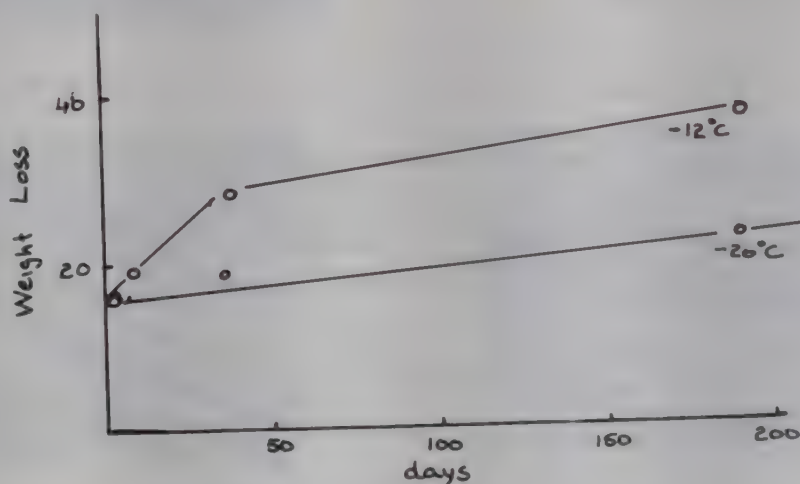
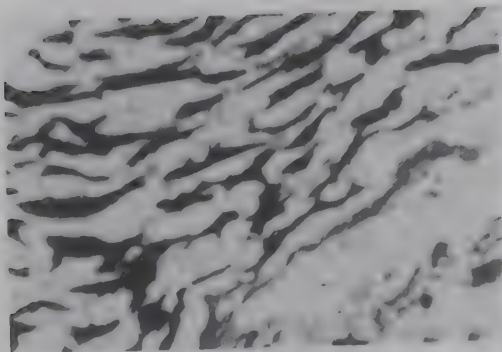
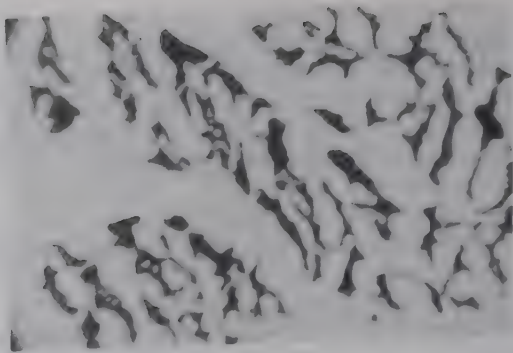


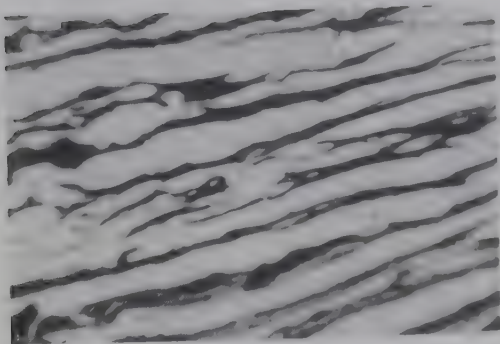
Figure 2: Weight loss on WHC test as a function of storage temperature and storage time



a

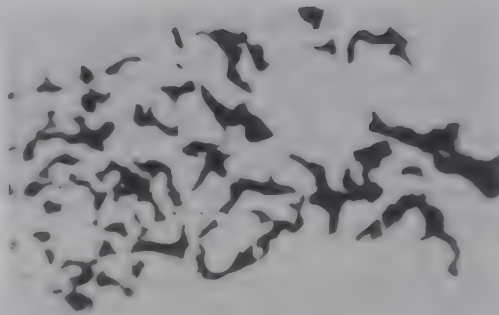


b



c

200μm

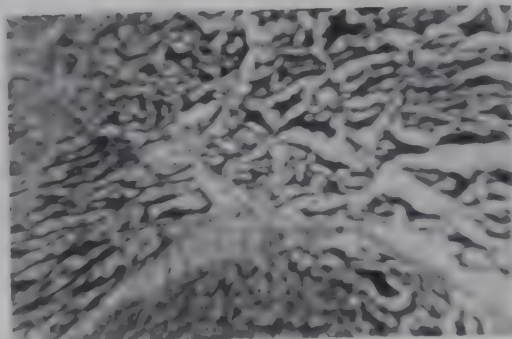


d

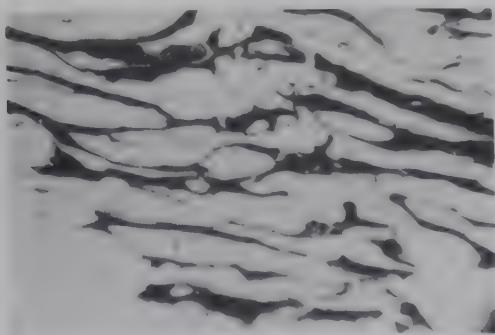
Figure 3: LM of samples IFF after various treatments.

- (a) -12° 1mo. stored LS
- (b) -12° 1mo. stored XS
- (c) -12° 6mo. stored LS
- (d) -12° 6mo. stored XS
- (e) -12° 6mo. stored AMBIENT FIX

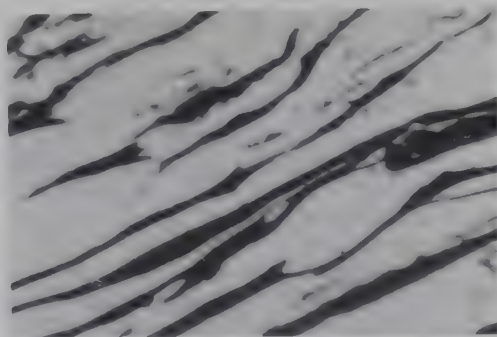
LS - Longitudinal section
XS - Cross section



e



a



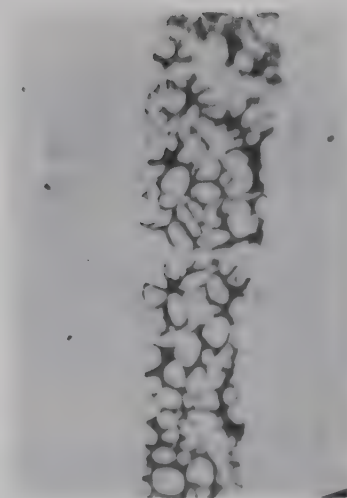
b

Figure 4: LM of samples IFF after various treatments.

- (a) -20° 3mo. storage LS
- (b) -20° 6mo. storage LS



a

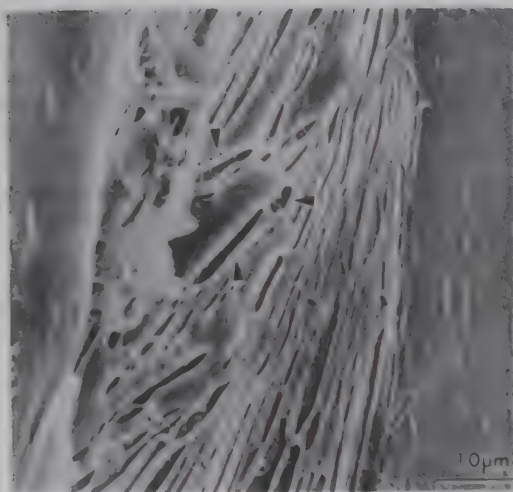


b

200 μm



c



d

Figure 5: Microscopy of samples IFF after various treatments.

- (a) SEM of sample IFF at -20°C after 10 days storage.
- (b) LM of sample IFF at -20°C after 3 months storage.
- (c) SEM of sample IFF at -5°C after 18 days storage.
- (d) detail of (c), from area marked C.

table 1

temperature	content of 100 ml fixative		
-20°C	34% DMSO	2.4% cacodylate	3% glutaraldehyde
-12°C	24% DMSO	2.4% cacodylate	3% glutaraldehyde
-5°C	DMSO	2.4% cacodylate	3% glutaraldehyde

Composition of isothermal freeze fixative

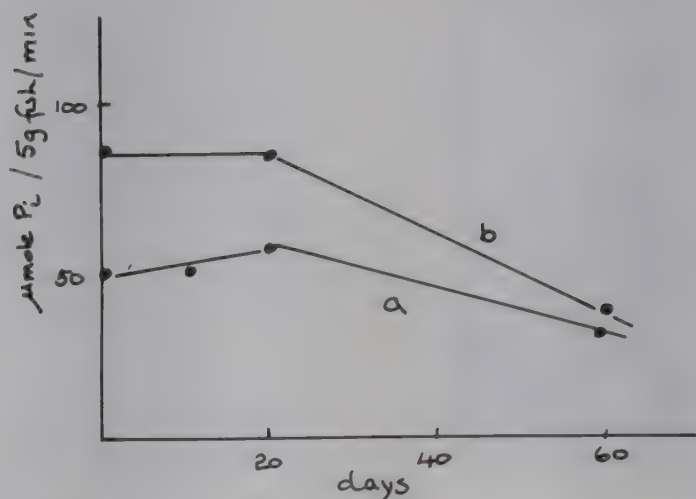


Figure 6: ATPase activity of myofibrillar fraction after storage at -20°C for varying periods

The data presented show an increasing loss of water holding capacity with frozen storage time, a decrease in protein extractability, and a slight loss in ATPase activity. The viscosity does not change significantly, remaining around 15000 cp. The pH of individual fish lies in the range 6.1-6.6. This, too, does not change during our storage period. Storage is continuing to follow these trends, and our data base is being expanded. We hope, when the study is complete, to be able to better characterize the structure-status relationship in frozen fish tissues. It is our goal to define more clearly the optimum freezing and frozen storage conditions for different fish species, and to understand better the reasons for any differences.

REFERENCES

- Eide, O., T. Borresen and T. Strom. 1982. J. Food Sci. 47:347
- Fiske, C.H., and Y. Subbarow. 1925. J. Biol. Chem. 66:375
- Gornall, A.G., C.J. Bardawill, and M.M. David. 1949. J. Biol. Chem. 177:751
- Groninger, H., J.W. Hawkes and J.K. Babbitt. 1983. J. Food Sci. 48:1388
- Hashimoto, K., S. Watanabe, M. Kono, and K. Shiro. 1979. Bull. Jap. Soc. Fish. 45:1435
- Lampilla, L., V. Mohr and D.S. Reid. 1985. J. Food Microstructure, in press

Acknowledgement

The work described received support from NOAA National Sea Grant College, Program under grant number NA 80AA-D-00120 AS, California Sea Grant College Program Project R/F 95

ETUDES DE L'ENTREPOSAGE DU POISSON A L'ETAT CONGELE

RESUME: L'effet de la congélation et de l'entreposage à l'état congelé des poissons de l'espèce *Sebastes* a été évalué à -5°C , -12°C , et -20°C . Une technique nouvelle, la fixation isothermique à l'état congelé, est utilisée. Les tissus congelés ont été fixés par cette méthode avant examen au microscope électronique à balayage ou au microscope optique. On peut voir les effets des cristaux de glace. L'effet de l'entreposage à l'état congelé sur les propriétés biochimiques a été évalué aussi.

DISCUSSION

M.J. NEWMAN (U.K.) - Can you use the techniques described to indicate whether fish has been stored at any time during the storage period at a temperature warmer than specified? For example, if fish should be stored at -26°C or -29°C , can you say whether the fish has been subjected to a period of storage at -21°C , -18°C or -15°C , and if so, for how long?

D.S. REID - The short answer is that we do not know. However, one of our objectives is to correlate, if possible, frozen structure with temperature history. Different levels on a core relate to different histories even on the same sample. We are trying to determine ice crystal number and size distribution for a large number of samples of known history. If consistent correlations emerge then we will have a tool to do just what you ask.

L.C. DE MATOS (Portugal) - According to your graph showing water losses at different temperatures (not in text) the water loss is highest at -5°C . Japanese studies on superchilling indicate that for certain species, if the freezing temperature is not lower than -2.6°C the percentage of ice in the cell does not exceed 30% and there is no damage to the cell walls. At that temperature, therefore, the water loss should be a minimum - do you agree with this?

D.S. REID - At temperatures higher than -3°C , or so, when the ice content is less than 30% in a frozen system, freezing damage is slight. However, below -4°C ice content increases rapidly and freezing damage can be significant. Superchilling has been shown to be effective for fish and for fruit and vegetables. Presumably, unfrozen matrix concentrations are the key. The increasing water loss during storage at -5°C compared to -20°C reflects, too, the greater rate of change of tissue status at -5°C .

P. HOWGATE (U.K.) - Do you intend to examine the samples by transmission electron microscopy (TEM) since it is possible to see the effects of storage time and storage temperature in thin sections?

D.S. REID - We have prepared blocks of samples during this study and we are now engaged in the process of examining representative samples by TEM. Our choice of blocks to examine is based on the optical micrographs and their indication of changing frozen structures.

L. HAN-CHING (France) - Do you intend to use TEM techniques to observe the effects of growth of crystals on the structure of protein matrix since, in your paper, you show that damage by ice crystals results in a loss of biochemical quality due to the protein change?

D.S. REID - All samples are fixed in a manner appropriate for TEM and, on the basis of the light micrographs, we are selecting blocks for investigation by TEM. When ice crystals are large TEM is not suitable for their study. TEM, as you indicate, allows us to look at the unfrozen matrix which contains the protein and other components.

T. BORRESEN (Denmark) - 1) What are the actual cooling rates in fast and slow freezing?

2) Are the ice crystals formed during fast freezing smaller than at slow freezing?

D.S. REID - 1) We measured the cooling rates at various locations, including the thermal centre, but I do not have these data with me. Slow freezing takes 24 hours and fast freezing takes 15 to 30 minutes between 0°C and -20°C .

2) The ice crystals formed initially during fast freezing are smaller than those formed on slow freezing. During storage they increase in size as the photomicrographs show.

EFFECT OF DIFFERENT HYDROCOLLOIDS ON THE STABILITY OF FROZEN STORED MINCED FILLETS OF WHITING AND COD.

D.J.B. da Ponte, J.P. Roozen and W. Pilnik.
Agricultural University, Department of Food Science
Wageningen, The Netherlands.

INTRODUCTION

The promising prospects of mechanical deboned fish are contrasted with several problems not yet completely solved. Normally the minced fish is less stable in frozen storage than the fish or the fillets. Changes in texture, flavour and water holding capacity are reported to be accelerated in the minced fish (Howgate, 1983; Froning, 1981).

Several attempts have been made to protect the functional properties of minced fish during frozen storage. Additions of sucrose, sorbitol and polyphosphates have been used to improve the quality of frozen minced fish in Japan (surimi) (Lee, 1984; Suzuki, 1981). However these additions may cause changes in taste which can lower the acceptance by occidental consumers. Recently the use of hydrocolloids was proposed as additives to minced fish (Codex Alimentarius Commission, 1981/1983).

In our experiments we added various hydrocolloids to minced fillets of whiting and cod and studied their effects on texture and water holding capacity during 3 months of frozen storage.

EXPERIMENTAL

Two lots of whiting fillets were purchased from the Institute for Fishery Products TNO, IJmuiden. One was caught on the 26th of January (1) and other on the 24th of October (2) 1983. The fillets were minced and then pre-freeze-dried hydrocolloids (High methoxy esterified pectin (H.P.), Low methoxy esterified pectin (L.P.), different types of alginates (Kelcogel, Kelcosol and Kelcocolloid), Kappa and Iota carrageenan, Locust bean gum (L.B.G.) and Xanthan gum, were added (5g/Kg). One lot of fillets of cod, bought in the local market of Wageningen on the 25th of January 1984 (3), was minced and Kappa, Iota and Lambda carrageenan and different types of carboxymethyl celluloses (CMC 705, CMC 1505, CMC 2205 and CMC 2805) were added (5g/Kg).

Hydrocolloids used were obtained from:

- Low esterified pectin and High esterified pectin from Obi-Pectin (Bischofszell, Switzerland).
- Potassium Kappa carrageenan (HF 55758-63) and Iota carrageenan (HF 33895-96) from Copenhagen Pectin Factory Ltd. (Denmark).
- Lambda carrageenan, Viscarin 402, (Batch 321403), from Marine Colloids Division, FMC Corporation, (Springfield, New Jersey, U.S.A.).
- Kelcogel HV, Kelcosol and Kelcocolloid HVF from Kelco/AIL London (England).
- Xanthan gum, Keltrol F (KTLF-67203A), from Kelco (New Jersey, U.S.A.).

I.I.F. - I.I.R. - Commissions C2,D3 - Aberdeen (United Kingdom) - 1985-4

--Locust bean gum, Meyprodin 200, from Meyhall Chemical AG (Switzerland).
 --Carboxymethyl cellulose, Akucell AF 705, AF 1505, AF 2205 and AF 2805 from Enka bv, Industrial Colloids (Holland).

Samples of the minced fish with the different additions were blast frozen and were stored at -18°C for about 3 months. They were evaluated at regular intervals for (Da Ponte et al., 1985):

--Water holding capacity of the raw material (W.H.C.R.)-15g of minced fish were centrifuged at 27750g for 30 minutes. The supernatant was discarded. Four replicates were done for each sample.

$$\text{W.H.C.R.} = \frac{\text{Residue}}{15\text{g}} \times 100\%$$

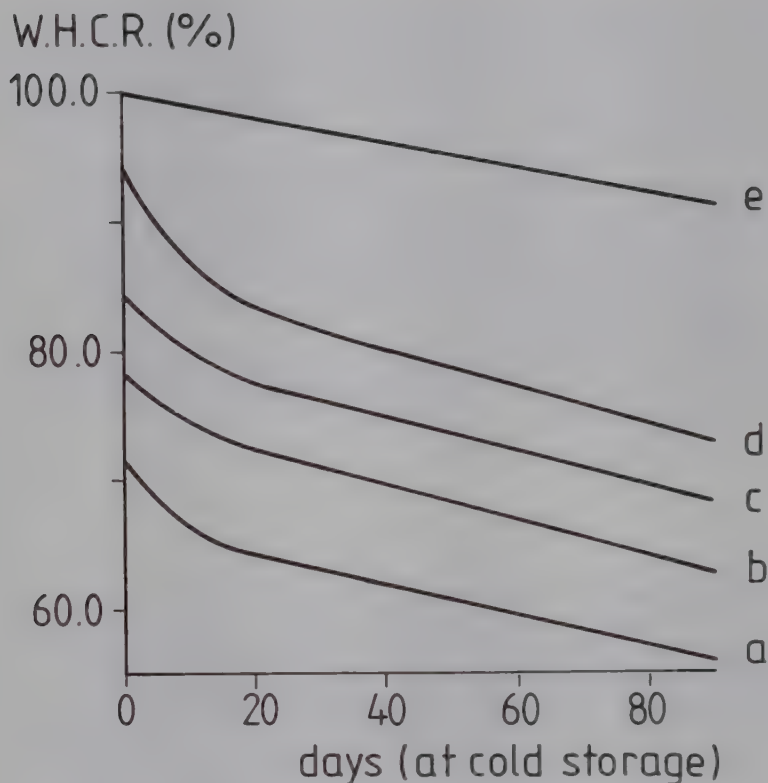


Fig.1 Effect of time and different hydrocolloids on W.H.C.R. of frozen stored minced fish.

- a.- Blank(1), L.P.(1), H.P.(1), K-carrageenan(1), Kelcocolloid(1), Blank(2), Blank(3) and K-carrageenan(3).
- b.- L.B.G.(2) and CMC 705(3).
- c.- Kelcogel(1), Kelcosol(1), 1-carrageenan(2), L.B.G.+Xanthan(2), 1-carrageenan(3), CMC 1505(3) and CMC 2205(3).
- d.- λ -carrageenan(3) and CMC 2805(3).
- e.- Xanthan(2).

--Cook drip loss (C.D.L.)-22g of minced fish were packed in 25ml beakers and cooked for 1 hour at 100°C. The cooked fish cake was poured out on 2 layers of filter paper and kept at room temperature for 1 hour. Five replicates were done for each sample.

$$\text{C.D.L.} = \frac{22\text{g-Fish cake weight}}{22\text{g}} \times 100\%$$

--Texture analysis:a.-Shear stress (S.S.) analyses were carried out in a Kramer Shear Press.75g of the raw minced fish were used. Two replicates were done for each sample.

b.-Compressive strength (C.S.) was determined in an overload dynamic apparatus. The fish cakes obtained from the C.D.L. determination were used. Five replicates were done for each sample.

RESULTS AND DISCUSSION

During 3 months of frozen storage at -18°C, W.H.C.R. decreased about 12% in all treatments (Fig.1). With few exceptions, Kappa carrageenan, pectins

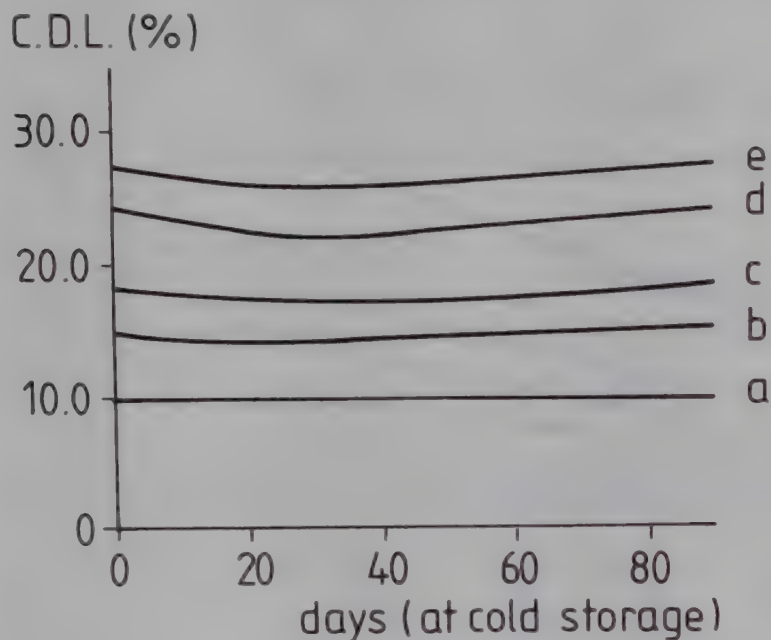


Fig.2 Effect of time and different hydrocolloids on C.D.L. of frozen stored minced fish.

- a.- Xanthan(2).
- b.- Kelcogel(1), L.B.G.+Xanthan(2) and CMC 2805.
- c.- K-carrageenan(1), Kelcosol(1), 1-carrageenan(2), L.B.G.(2) and CMC 2205(3).
- d.- Blank(1), L.P.(1), H.P.(1), Kelcolloid(1), Blank(2), K-carrageenan(3), λ-carrageenan(3), 1-carrageenan(3) and CMC 1505(3).
- e.- Blank(3) and CMC 705(3).

(L.P. and H.P.) and one alginate (Kelcocolloid), the W.H.C.R. of the additions were significantly higher than the respective Blanks. Xanthan presented the highest W.H.C.R. immediately followed by the hydrocolloids with the highest viscosities (CMC 2805, CMC 2205, CMC 1505, Kelcogel and Kelcosol). The high W.H.C.R. presented by Lambda and Iota carrageenan is probably due to the high capacity of their hydrated particles to retain water. The combination of L.B.G. and Xanthan showed also high W.H.C.R.. This combination is well known for its formation of strong cohesive and thermoreversible gels (Cottrell et al., 1980; Mc Neely and Kang, 1973).

C.D.L. was almost constant during the 3 months of frozen storage (Fig.2). Xanthan presented the lowest C.D.L. followed by Kelcogel, the combination of Locust bean gum and Xanthan and the carboxymethyl cellulose with the highest viscosity (CMC 2805). In the Kappa and Iota carrageenan additions, the formation of a gel explains their differences when compared with the respective Blanks.

The hydrocolloids used had a big impact on the S.S. and C.S. (Figs. 3 and 4). The samples with Xanthan were very soft, like a paste or a pudding, and showed the biggest resistance to toughening during the 3 months of frozen storage. Except Kappa carrageenan, all the additions presented less increase of C.S. and S.S. than the Blank.

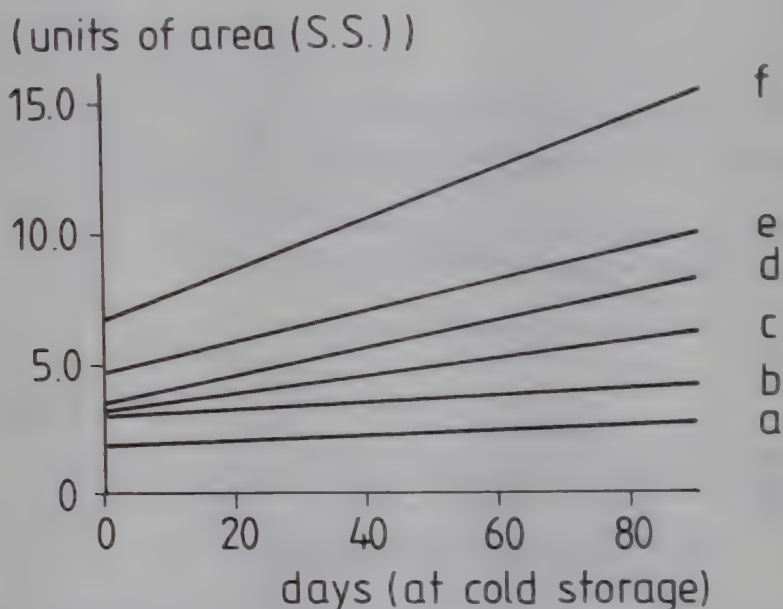


Fig.3 Effect of time and different hydrocolloids on S.S. of frozen stored minced fish.

- a.- Xanthan(2) and λ -carrageenan(3).
- b.- L.B.G.+Xanthan(2), λ -carrageenan(3), CMC 2205(3) and CMC2805(3).
- c.- Kelcogel(1), Kelcosol(1), λ -carrageenan(2), CMC 705(3) and CMC 1505(3).
- d.- L.B.G.(2), Blank(3) and K-carrageenan(3).
- e.- L.P.(1), H.P.(1), Kelcocolloid(1) and Blank (2).
- f.- Blank(1) and K-carrageenan(1).

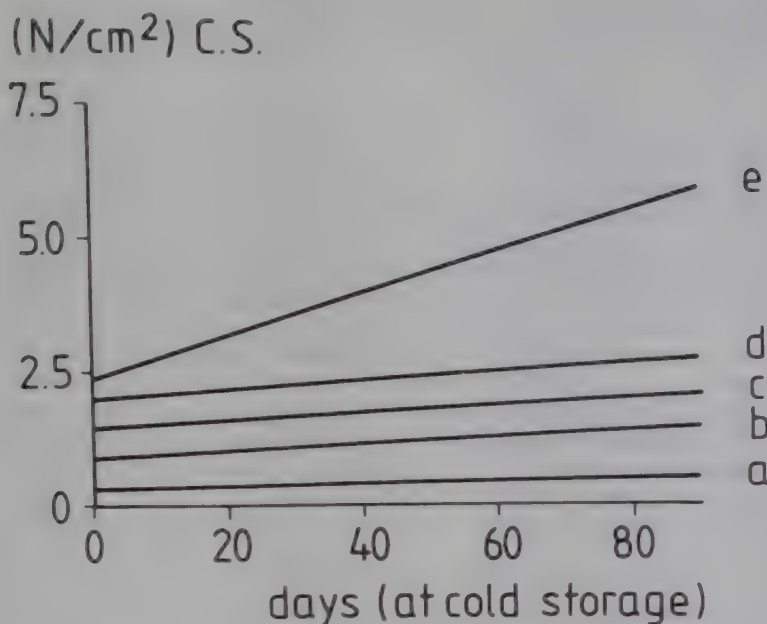


Fig.4 Effect of time and different hydrocolloids on C.S. of frozen stored minced fish.

- a.- Xanthan(2).
- b.- L.B.G.+Xanthan(2), CMC 2205(3) and CMC 2805(3).
- c.- L.P.(1), Kelcogel(1), Kelcosol(1), λ -carrageenan(3), CMC 705(3) and CMC 1505(3).
- d.- H.P.(1), Kelcocolloid(1), 1-carrageenan(2), L.B.G.(2) and 1-carrageenan(3).
- e.- Blank(1), K-carrageenan(1), Blank(2), Blank(3) and K-carrageenan(3).

The carboxymethyl celluloses used can be blended directly with the minced fish without the pre-freeze-drying operation.

CONCLUSIONS

The hydrocolloids used change the texture and water holding capacity of the minced fillets of whiting and cod during frozen storage. In some hydrocolloids (alginates, carboxymethyl celluloses and Xanthan), the viscosity is the main factor which influences these parameters. In other hydrocolloids (carrageenans, Locust bean gum and combination of locus bean gum and Xanthan), the formation of a gel and the capacity of holding water as hydrated particles are the main reasons for the changes observed.

To a certain extent it is possible to control and stabilize the texture and water holding capacity of the minced fillets of whiting and cod. This may be useful for the development of products from minced fish.

REFERENCES

- Codex Alimentarius Commission 1981/1983. Report of the fourteenth/fifteenth session of the codex committee on fish and fishery products.
- Cottrell, I.W., Kang, K.S. and Kovacs, P. 1980. Xanthan gum. In Handbook of water soluble gums and resins, (R.L. Davidson ed.) chapter 14. Mc Graw-Hill Book Company. New York.
- Da Ponte, D.J.B., Roozen, J.P. and Pilnik, W. 1983. Effects of additions on the stability of stored minced fillets of whiting. I-Various anionic hydrocolloids. *J. Fd Quality* (in press).
- Froning, G.W. 1981. Mechanical deboning of poultry and fish. *Adv. in Food Res.* 27, 109-147.
- Howgate, P. 1983. Quality of deboned fish flesh. In *Sensory quality in foods & beverages*, (A.R. Williams, R.K. Atken eds.) pp. 361-371. Ellis Horwood Ltd. Chichester.
- Lee, C.M. 1984. Surimi process technology. *Food Technology* 41, 69-80.
- Mc Neely, W.H. and Kang, K.S. 1973. Xanthan and some other biosynthetic gums. In *Industrial gums*, (R.L. Whistler ed.) pp. 473-497. Academic Press. New York.
- Suzuki, T. 1981. Frozen minced meat (surimi). In *Fish and krill proteins: processing technology*, (T. Suzuki ed.) pp. 113-147. Applied Science publishers, Ltd..

INFLUENCE DE DIVERS HYDROCOLLOIDES SUR LA STABILITE DE FILETS HACHES DE MERLAN ET DE CABILLAUD CONSERVES A L'ETAT CONGELE

RESUME : Des pectines, des alginate, des carraghénanes, des carboxyméthyl-celluloses, de la xanthane, de la gomme de caroube et de la combinaison de la Xanthane et de la gomme de caroube ont été employées comme additifs (5g/kg) dans des filets hachés de merlan et de cabillaud. Des échantillons soumis à ces traitements ont été entreposés à -18°C pendant 3 mois et la capacité de rétention d'eau et la texture ont été évaluées à des intervalles réguliers.

Les auteurs ont observé des différences remarquables de la texture et de la capacité de rétention d'eau. A l'exception de la cappa-carraghénane et des hydrocolloides à une viscosité relativement faible, l'addition de ces additifs a amélioré la capacité de rétention d'eau et a réduit les processus de durcissement au cours de la congélation.

THE STORAGE LIVES OF VARIOUS FROZEN MINCES AND FILLET OF COD COMPARED WITH FIVE OTHER GADOID SPECIES

K. W. Young, A. Craig, P. Howgate, G. L. Smith and K. J. Whittle
Torry Research Station, Aberdeen (United Kingdom)

1. INTRODUCTION

Increasingly, fish processors have to use available fish resources more fully as a result of economic factors and pressures on supplies of fish. Over the last 10 to 15 years much greater use has been made of less usual white fleshed resources, eg Alaska pollack, various hakes, blue whiting and whiting, and advances in fish processing machinery to increase yield. Machines which separate edible flesh from skin and bone are used widely to enhance yields and process small sizes of fish. The recovered mince is stored as frozen blocks which are often traded internationally as a commodity for processing elsewhere.

Flesh can be recovered from fillets, trimmings from filleting, split fish, headed and gutted fish and frames or skeletons. Understandably, such minces vary both in overall quality and the rate at which they deteriorate during storage /1, 2, 3/. The extent of the variability has not been established fully, but clearly is a matter of some concern to processors and manufacturers with respect to potential shelf life. Preliminary results of our comparative studies over one year on the cold storage stability of blocks made from different types of minces derived from a number of gadoid species, and based on assessments of 'acceptability', have been reported elsewhere /3/. Here, we report for comparison, the complementary studies on cod, the species most widely used in the UK.

2. MATERIALS AND METHODS

2.1 Fish supplies: Three tonnes, gutted, iced cod (Gadus morhua), of freshness score not less than eight on the Torry wet fish scoring system, were purchased at Aberdeen Fish Market by a local fish processor. The fish were caught on two grounds, Bergen (80%) and West of Shetland (20%).

Details of the supplies, preparation and storage of the other gadoid species discussed later, eg haddock (Melanogrammus aeglefinus), whiting (Merlangius merlangus), South African hake (Merluccius capensis), Alaska pollack (Theragra chalcogramma) and blue whiting (Micromesistius poutassou), are given elsewhere /3/.

2.2 Processing: Initial handling and preparation was carried out by the processor. The cod were headed (Baader 421), filleted and skinned (Baader 184/51 combination), and the fillets trimmed by hand, by deep 'J-cutting'. The J-cuts were halved to give the equivalent of V-cut trimmings and belly flaps. The latter were bulked with other trimmings from the fillets, eg fin. The filleting machine also removed the neural spines from the frames; the hemal spines were removed by hand and the spines bulked.

Subsequent preparation was carried out at the laboratory. The fillets, trimmings and spines were iced and stored in a chill room (2-4°C) overnight; the flesh was recovered from the trimmings and spines the next day (Baader 694; drum perforations, 5 mm diameter).

2.3 Block preparation: The compositions were:

A/C - Fillet	skinless, J-cut fillets only
P - Fillet & V-cut mince	fillets as above (80%) plus mince (20%)
Q - V-cut mince	V-cut trimmings only
R - Belly flap mince	belly flaps and other fillet trimmings eg fin
S - Spine mince	neural hemal spines (62.5%) and J-cut trimmings (37.5%)

The blocks (nominally 7.4 kg) were prepared and frozen in the usual commercial fashion /4/; and sawn longitudinally into thirds but no additives were used. Each portion was double wrapped in aluminium foil prior to frozen storage.

2.4 Storage: Sufficient samples were stored at -15°C (C, P to S) and -30°C (A, P to S) to make periodic assessments over a one year period.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

2.5 Tasting: At each assessment, the samples were cut into sticks (9 x 2 x 1 cm) coated with batter and breadcrumbs (Stein Mini Breader MB 1) and kept frozen until required for tasting within 24 hours. The frozen fingers were fried in deep fat /3/ and not more than 5 samples, coded with three-digit random numbers, were presented to a panel at a session.

Panels of 42 tasters were drawn from a pool of 110 scientific and support staff of the research station and visiting workers, to assess samples without formal training using a 9-point hedonic scale.

After tasting each fish finger sample, the panellists were asked to select from the list of like and dislike descriptions below, a number which fitted their impression of each sample in terms of OVERALL ACCEPTABILITY:

- | | | |
|-------------------|----------------------------|----------------------|
| 9 like extremely | | 4 dislike slightly |
| 8 like very much | 5 neither like nor dislike | 3 dislike moderately |
| 7 like moderately | | 2 dislike very much |
| 6 like slightly | | 1 dislike extremely |

2.6 Statistical analysis: The change in median hedonic score with time was estimated by least absolute deviations linear regression /5, 6/. This was chosen because it is the regression analogue of the median and is not dependent on assumptions of normally distributed residuals. Its use with sensory data is discussed by Smith /7/.

The percentage liking defined as the proportion of assessors scoring 6 or more on the scale plus half of those scoring 5 was also used as an index of panel liking. Changes in percentage liking were estimated by least squares. Significance tests were used to investigate differences between the lines so obtained (see for example Snedecor and Cochran /8/).

3. RESULTS AND DISCUSSION

Figure 1 is a plot of the changes in the medians of hedonic scores over one year for the cod samples (C, P to S) stored at -15°C and, for comparison, the cod fillet (A) stored at -30°C . The variability in the data is shown clearly, but we have assumed that linear relationships describe the data best and the lines of best fit are superimposed. The observed initial acceptability of the spine mince (S) is one score unit higher than the value at the intercept of the fitted line, perhaps suggesting that the rate of change may be much higher for this mince in the initial stages of storage. More results would be required during this period to test the hypothesis. However, the rate of dimethylamine production, measured more frequently on samples of (S), was much more rapid during the first 5 or 6 weeks (W M Laird, personal communication) as Laird *et al.* /9/ had shown previously for frame minces. The spine mince was not liked from about the eighth week of storage.

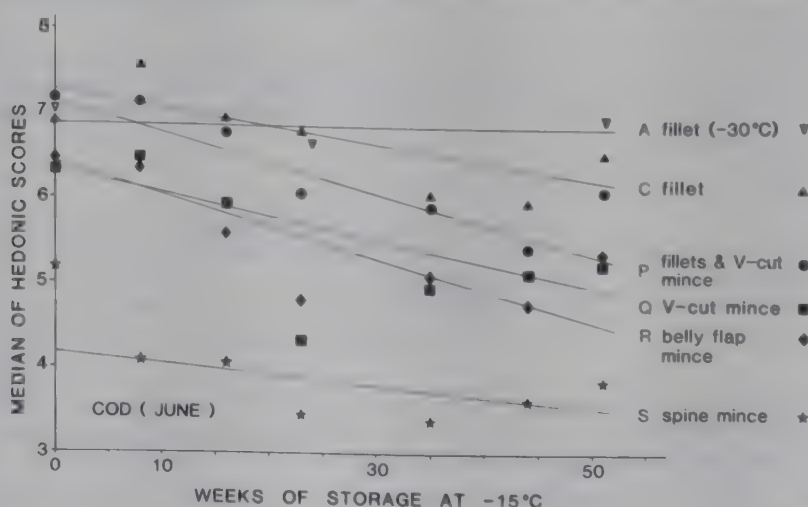


Fig. 1

A plot of medians of hedonic scores against storage time at -15°C for various cod fillet and mince preparations.

The line A is based only on three points. Nevertheless, there is little change over the storage period and the three values are well within the variation in median hedonic rating found for six similar trials /3/ over one year at -30°C on cod fillet blocks prepared from fish obtained at various times of year.

The ordering by panellists at each assessment point of the acceptability of the products stored at -15°C is remarkably consistent throughout the storage period of one year: fillet (C) > fillet plus V-cut mince (P) > V-cut mince (Q) and belly flap mince (R) > spine mince (S). This is similar to observations made in the earlier trials /3/ with other species. The acceptability of all these samples tends to decline slowly with time, but similar cod samples (A, P to S) stored at -30°C showed little or no change although the same consistent ordering of acceptability with respect to type of mince was observed.

The regression lines describing the change of percentage liking with storage time at -15°C are shown in Figure 2 (COD) with the datum points omitted for clarity, so that trends can be compared for each cod sample (C, P to S). These results can be compared directly with those of five other gadoids (calculated from the data of Whittle *et al.* /3/), shown in Figure 2 on the same scale. In all cases, cod fillet (A), processed at the same time as the test species but stored at -30°C , is included as a benchmark and clearly shows little change. The stability of the minces (D, E and Q, R, S) from the respective species stored at -15°C can be compared with fillets (C) from the same species and batch of fish.

Minced, whole cod fillet (B) stored at -30°C is also included for reference (except in the case of cod). It was both as acceptable and as stable as fillet (A). This observation was consistently confirmed in six completely independent, one year, storage trials carried out over an interval of four years. Each was based on cod caught in the North Sea between December and June, thus covering substantial seasonal variation. It was reasonable to pool all the datum points for A (66) and B (60); 90% fell between 72 and 92% liking. The same regression line (zero slope and intercept 83% liking) described either A or B over one year's storage. Samples of A stored at -30°C for longer periods up to 4 years continued to show no significant change, all plotting within 6% liking points of the regression line. In contrast, samples of B similarly stored showed values up to 25% liking points lower than the regression line, but there were insufficient numbers of assessments to determine whether the downward trend was significant.

The cod samples (C, P, Q, R, S) all deteriorated at similar rates at -15°C (slopes 23 to 32), but were ordered in three distinct and significantly different groups, with % liking in the order: fillet (C) and fillet plus V-cut mince (P) > V-cut mince (Q) and belly flap mince (R) > spine mince (S), much as might be anticipated. Although addition of 20% V-cut mince to the fillets tended to reduce % liking and enhance the rate of deterioration, the differences were small and not statistically significant.

Comparison with the results for the five other gadoid species at -15°C (Figure 2) shows that spine (S) and headed and gutted (H & G) minces (E) are liked least. Initially, the percentage of tasters liking ranged between 35 and 45% (cod, whiting, Alaska pollack) or 50 to 61% (haddock, hake, blue whiting), but the latter group show more rapid rates of deterioration (slopes 37 to 42, cf Table I). The fillets of the species (C) are liked most in every case. Initially, they range between 75 to 93%, except hake (65%), but rates of deterioration are variable (Table I), showing little or no change for haddock, whiting and Alaska pollack, a rate for blue whiting (slope 23) similar to cod, but a faster rate for hake (slope 31). The various minces from fillet trimmings (Q, R), block fillet (D) and single fillet (D), occupy the middle ground in terms of liking, initially ranging between 60 to 76%. Rates of deterioration fall into two groups, a larger, slower group of four species (slopes 25 to 27), with hake (42) and haddock (49) showing appreciably faster rates. Table I summarises the rates of deterioration and the initial percentage liking.

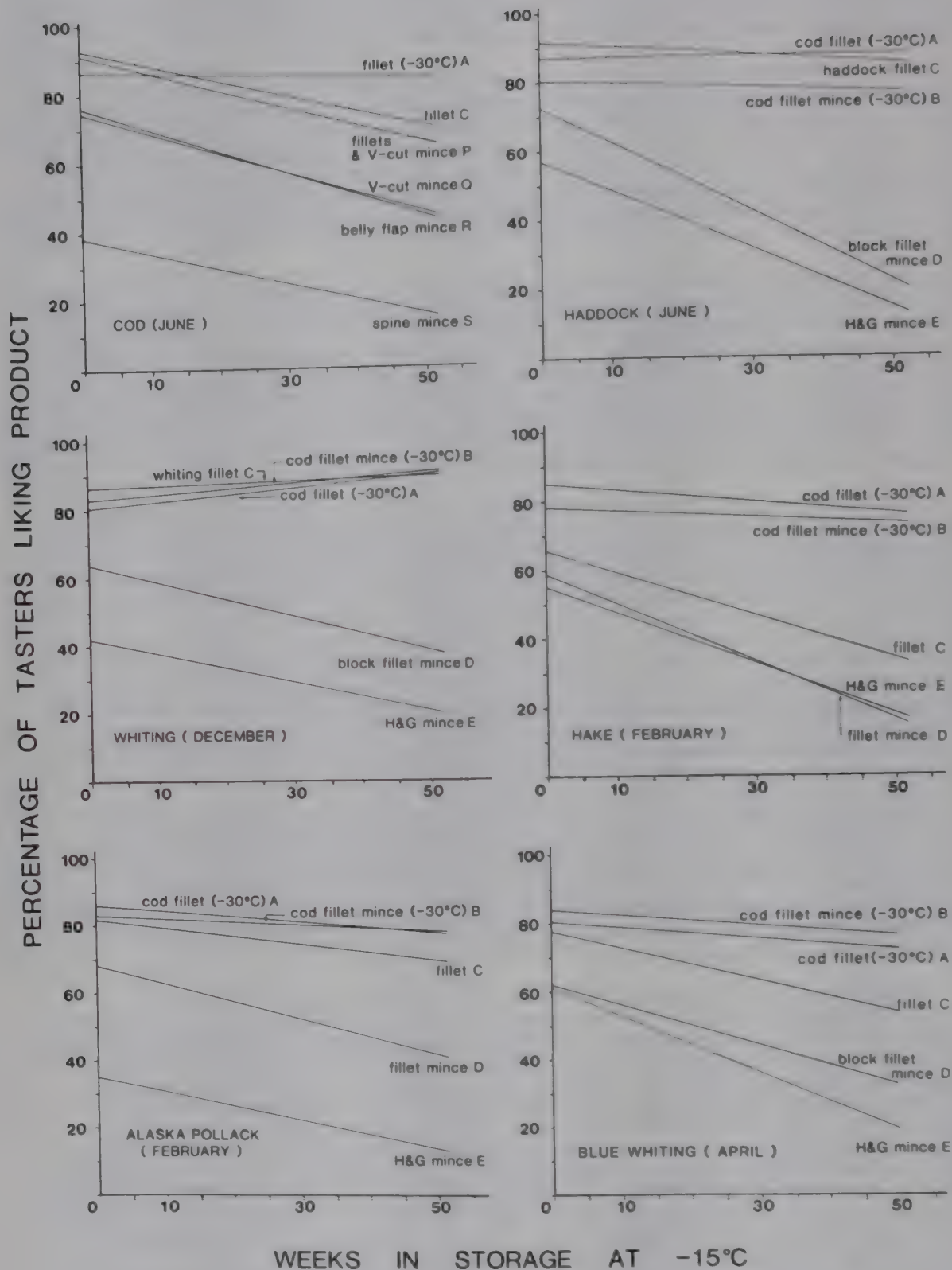


Fig. 2

Comparison of changes in percentage liking during storage at -15°C of fillet and mince preparations from six gadoid species.

TABLE I

Initial percentage liking, estimates of shelf life and rates of deterioration compared for frozen fillet or mince blocks stored at -15°C

Block Types	Initial % Liking	Cod (June)	Haddock (June)	Whiting (December)	Hake (February)	Alaska Pollack (February)	Blue Whiting (April)
Skinless C Fillet	65-93	50 ¹ **2	>52 *	>52 *	0 **	49 *	16 **
Fillet P Plus V-cut Mince	91	41 **					
V-cut Q Mince	75	9 **					
Belly R Flap Mince	75	10 **					
Block D Fillet Mince	62-72		2 ***	0 **			0 **
Single D Fillet Mince	60-68				0 ***	0 **	
H & G E Mince	53-61		0 ***	0 **	0 ***	0 **	0 ***
Spine S Mince	40	0 **					

1 Shelf lives are based on the regressions shown in Figure 2.

2 Rates of deterioration measured as loss of percentage liking per 50 weeks are grouped as follows: negligible or slow, <15, *; medium, 15 to 30, **; fast, >30, ***.

4. SHELF LIFE

Estimates of the length of practical shelf life for a product depend on (i) method of assessment, (ii) selection of the critical level of 'quality' below which the sample is rejected, (iii) temperature of storage, (iv) quality at the beginning of storage, (v) rate of deterioration during storage. For this discussion, 70% liking was selected as the critical level. Figure 2 shows that all the frame (S) or H & G minces (E) and most of the fillet minces (D) have NO shelf life at -15°C by this criterion, because the initial values of percentage liking are below 70%. The remaining mince samples and the fillet samples from blue whiting and hake have a short shelf life; initial values range between 70-80% and rates of deterioration are medium to fast. Most fillet samples (C), and the mixed fillet/mince sample (P), together with all the samples (A to C and P to R) stored at -30°C , have shelf lives of about one year or longer and show medium or slow rates of deterioration. Table I also summarises the estimates of shelf life for the fillet and mince preparations from the six gadoid species. It must be emphasised that the estimates are for frozen commercial-type blocks (without additives), stored at -15°C and assessed by measuring the acceptability of fish fingers manufactured from the blocks at the end of the experimental storage periods.

As noted earlier, variability is high in the hedonic data although the ordering of samples at each assessment is remarkably consistent. Consequently, much scatter about the regression lines gives rise to large errors in the estimates of slopes, intercepts and shelf lives. Linearity can only be assumed for the period covered by the data, and so it is not possible at present to extrapolate to values of shelf life beyond one year at -15°C where appropriate. However, storage of cod samples A and B at -30°C was extended up to 4 years as discussed earlier. In view of the few samples assessed so far, only tentative estimates of shelf life can be made of more than 200 weeks and more than 60 weeks for cod fillet blocks (A) and cod fillet mince blocks (B) respectively, when stored at -30°C . Further storage trials at -30°C and -20°C are being evaluated.

In general, storage temperatures as high as -15°C do not afford adequate protection for frozen minced fish blocks or even for fillet blocks from some of the species tested, whereas -30°C in the case of cod reduced the rate of deterioration to confer shelf lives of about one year or more.

REFERENCES

1. P. HOWGATE (1983) 'Quality of deboned fish flesh' in Sensory quality in frozen foods and beverages: definition, measurement and control. Edited by A. A. Williams and R. K. Aitken. Chichester: Ellis Harwood Ltd.
2. K. W. YOUNG and K. J. WHITTLE (1985) 'Colour measurement of fish minces using Hunter L , a , b values'. J. Sci. Food Agric., 36, 383-392.
3. K. J. WHITTLE, K. W. YOUNG, P. HOWGATE, A. CRAIG and G. L. SMITH (1983) 'Storage life of fish minces' in Thermal processing and quality of foods. Edited by Zeuthen *et al.* Elsevier Applied Science Publishers Ltd.
4. J. N. KEAY, R. M. STOREY and K. J. WHITTLE (1982) 'Other fish products' in Fish Handling and Processing (2nd Edition) edited by A. Aitken, I. M. Mackie, J. H. Merritt and M. L. Windsor, Edinburgh HMSO.
5. O. J. KARST (1958) 'Linear curve fitting using least deviations'. J. Am. Statist. Soc., 53, 118-132.
6. R. D. ARMSTRONG and M. T. KUNG (1978) 'Algorithm AS 132: Least absolute value estimate for a simple linear regression problem'. Applied Statistics, 27, (3), 363-366.
7. G. L. SMITH (1984) 'Statistical analysis of sensory data' in Sensory analysis of foods pp. 305-349 edited by J. R. Pigott, Elsevier Applied Science Publications Ltd, London.
8. G. W. SNEDECOR and W. G. COCHRAN (1980) Statistical methods (7th Edition) Iowa State University Press, Iowa, America.
9. W. M. LAIRD, I. M. MACKIE and J. R. REGENSTEIN (1981) 'Deterioration of frozen cod and haddock minces'. Bull. Int. Inst. Refrig. Annexe 1981-4, 395-400.

L'APTITUDE A L'ENTREPOSAGE DE DIVERS HACHIS ET FILETS CONGELES DE CABILLAUD COMPAREE AVEC CELLE DE CINQ AUTRES ESPECES DE GADOIDE.

RESUME : Des hachis de cabillaud, préparés à partir de coupes, de flancs et de déchets récupérés sur les arêtes, ont été décongelés en blocs de 7,4kg et entreposés 1 an à -15°C et -30°C . Des blocs de filets et des blocs de hachis de filets avec des chutes ont été préparés et stockés de façon similaire. A chaque contrôle périodique pendant l'entreposage, des échantillons congelés ont été préparés sous forme de bâtonnets de poisson et ont été appréciés par un large jury de dégustateurs non entraînés appliquant la méthode hédonique. Les résultats du jury pour les échantillons à -15°C , exprimés en pourcentage de préférence, étaient classés en décroissant de la façon suivante en fonction de la période de stockage : filets et hachis de filets et chutes (4:1) > à hachis de chutes et hachis de flancs > au hachis de déchets récupérés sur les arêtes; tous diminuent depuis le début de façon semblable. A -30°C tous les échantillons montrent peu de changement pendant toute la période de stockage par rapport à leur état initial. Tous ont obtenu un pourcentage d'acceptabilité de 70 % ou plus, sauf pour le hachis de déchets récupérés sur les arêtes qui n'a jamais atteint plus de 40 %.

L'estimation de durée d'entreposage dépend de la limite minimale choisie pour le pourcentage d'acceptabilité. Les filets et hachis de filets et chutes jugés sur la valeur normale de 70 % d'acceptabilité ont une durée d'entreposage de 1 an à -15°C . Sauf pour les hachis de déchets récupérés sur les arêtes, tous les échantillons ont une durée d'entreposage d'environ 1 an ou plus à -30°C . Les résultats sont comparés avec ceux obtenus pour le haddock, le merlan, le merlan bleu, la merluche d'Afrique du Sud et le lieu d'Alaska, ainsi qu'avec les modifications observées pour les filets de cabillaud et les hachis de filets entreposés jusqu'à 4 ans à -30°C .

DISCUSSION

A. MILLS (U.K.) - Is it possible that samples of mince can improve during storage ? From Figure 1 in the text of your paper it appears that the acceptability of v-cut mince and belly flap mince may be improving.

P. HOWGATE - The results from these trials are being reconsidered and it may well be that the texture of some products 'improve' in terms of consumer acceptability.

FROZEN STORAGE OF FISH AND FISH PRODUCTS IN JAPAN AND NOVEL DEHYDROFREEZING OF FISH

Tsuneo T. Kozima
Department of Food Technology and Engineering
Tokyo University of Fisheries
Tokyo, Japan

1. FROZEN STORAGE OF FISH AND FISH PRODUCTS IN JAPAN

Japan has a total annual fish catch of about 12 million metric tons. Domestic production and imports of frozen fish are increasing year by year, as shown in Table I.

TABLE I

Total Catch of Fish, and Production and Imports of Frozen Fish in Japan
('000 tonnes)

Year	Total Catch	Production of Frozen Fish	Frozen Production as % Total Catch	Imports
1971	9,908	2,632	26.6	323
1972	10,213	2,719	26.6	350
1973	10,763	2,982	27.7	472
1974	10,808	2,878	26.6	483
1975	10,545	2,906	27.7	538
1976	10,656	3,070	28.8	619
1977	10,757	3,164	29.8	721
1978	10,828	3,137	29.0	789
1979	10,590	3,154	29.8	891
1980	11,122	3,118	28.0	735
1981	11,319	3,608	31.9	877
1982	11,388	3,883	34.1	994
1983	11,967	3,915	32.7	1,042

Japan utilises many species of fish; the frozen production of the main species is shown in Table II. The amount of frozen fish increased rapidly with the modernisation of the food distribution system in Japan, while the storage temperature of frozen foods tended to be gradually lowered. This tendency towards lower storage temperatures must now be reconsidered to ensure that suitable storage times and temperatures are chosen to minimise energy usage. Current recommendations on suitable storage times and temperatures of frozen foods (TTT factors) may not be appropriate to Japan which has different food customs and food materials from other countries. Determination of suitable storage temperatures is required under practical conditions in Japan.

TABLE IV

Production of Main Species of Frozen Fish in Japan in 1983 ('000 tonnes)

Species	Production of Frozen Fish	Catches	Percentage of Catch Frozen
Herring	13	8	
Sardine species	1,300	4,082	31.9
Horse mackerel	48	179	26.8
Mackerel	344	805	42.7
Saury	179	240	74.6
Bonito	144	353	40.8
Tuna Species	232	357	65.0
Swordfish species	34	48	70.8
Salmon and trout	54	161	33.5
Flatfish species	102	258	39.5
Cod and pollack	195	1,539	12.7
Sand eel	101	120	84.2
Squid species	358	539	66.4
Whale meat	19		
Surimi	380		
Other fishes	297		
Shellfish	35		
Other marine animals	99		
TOTAL	3,921	8,681	35.6

The Japanese Association of Refrigeration has set up a committee to investigate suitable storage temperatures for frozen foods in Japan. This committee has compared the literature data on storage temperatures with data from investigations of the actual storage conditions in a hundred stores belonging to members of the Japanese Association of Refrigerated Warehouses, and with results of experiments on the relationships between storage time and temperature for the five most important Japanese coastal fish species and for frozen surimi.

Suitable storage condition for frozen fish and fish products in Japan are shown in Table III.

TABLE III

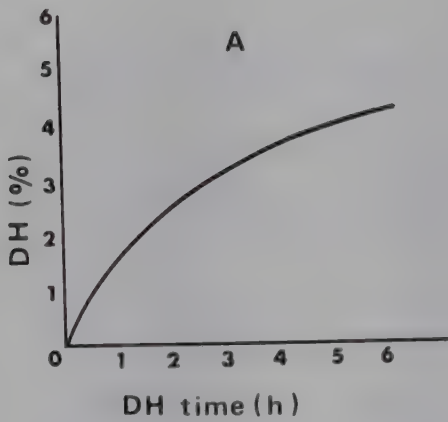
Suitable Storage Conditions for Frozen Fish and Fish Products in Japan

Species	Storage Temperature (°C)	Storage Time (Months)
Sardine	-18	6
	-23	12
Mackerel	-18	6
	-23	8
Saury	-18	6
	-23	12
Herring	-18 to -20	4 to 6
Cod	-18	4 to 6
	-20	8 to 9
	-23	9 to 10
Flatfish	-18	7 to 12
Horse mackerel	-18	12
Capelin	-18 to -20	4 to 6
Tuna	-30	3 to 6
Swordfish	-40	6
Bonito (eating fresh)	-30	6
	-40	6
Bonito (for processing)	less than -20	
Squid	-18	12
Octopus	-20	6
	-25	12
Salmon	-18	5 to 8
Trout	-23	10
Sea bream	-18	3 to 5
	-25	12
Surimi (pollack)	-23 to -25	6 to 12
Salmon roe, Cod roe (salted)	-18 to -22	6 to 12
Herring roe (product)	-14	6
" " (salted raw material)	-14	12
Salted fish	-23	6 to 10
Dried small sardine	-18 to -25	6 to 12
Dried squid		
Shrimp, crab	-18	6 to 12
	-25	12 to 15
Oyster	-18	5 to 9
Scallop	-23	9
Whale meat	-18	4 to 6
	-20	12
Dried wakame (salted)	-14	6
(<i>Undaria pinatifida</i>)		

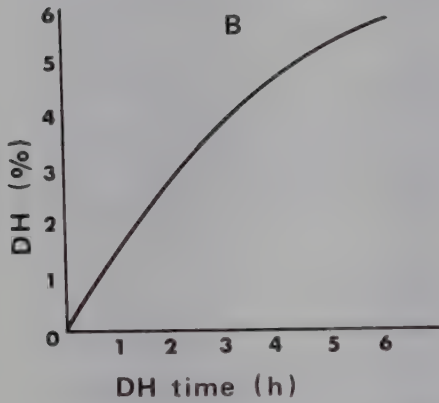
2. NEW DEHYDROFREEZING METHOD FOR FISH

Dehydrofreezing is a method of removing water from foods such as fish, meat and vegetables and then freezing. The dehydration is carried out with a dehydration sheet (contact-dehydration sheet) composed of a mixture of superabsorbent polymer and high osmotic pressure sugar hydrolysate covered with semipermeable plastic sheet.

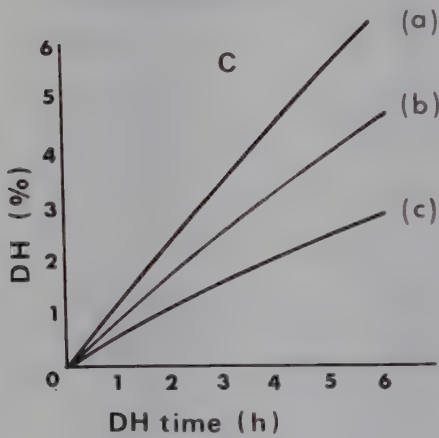
Figure 1 shows the dehydration rates obtained with the dehydration sheet for three species of fish.



A Salmon fillet dehydrated at 2°C after being dipped in 5% NaCl solution for one hour



B Codfish fillet dehydrated at 2°C after being dipped in 8% NaCl solution for one hour



C Halfbeak

	a	18°C
DH Temp	b	10°C
	c	2°C

Fig. 1 Dehydration (DH) rate of fish with dehydration sheet

Compared with their conventionally frozen counterparts, the advantages are:

- 1 Control of texture and moisture is better
- 2 drip losses are reduced
- 3 soft-flesh fish becomes firmer and does not break up; gaping does not occur
- 4 fish are easy to marinate.

Dehydrofrozen fish once thawed can be served in slices in typical Japanese fashion for fresh fish.

Tables 4 and 5 show various measurements on the dehydrofrozen fish.

TABLE IV

Quality of Dehydrofrozen Bonito and Yellow Tail

	Striped Bonito (Hagatuo)		Yellow Tail (Buri)	
Dehydration (Percentage)	+	-	+	-
	(2.6%)		(1.1%)	
Moisture (%)	66.9	66.3	74.4	75.0
VB-N (mg %)	13	13	13	12
pH	5.7	5.7	5.6	5.6
K Value (%)	28	26	6	6
TMA-N	trace	trace	trace	trace
Drip ml/100 g	1.7	3.4	3.8	4.7
Hardness (g/cm ³)	300	300	350	350
(Min ~ Max)	(270 ~ 390)	(250 ~ 420)	(290 ~ 410)	(290 ~ 450)
Shearing force (g)	200	180	370	380
(Min ~ Max)	(150 ~ 230)	(140 ~ 240)	(300 ~ 480)	(320 ~ 450)

TABLE V

Quality of Dehydrofrozen Mackerel (Masaba) Assessed by K Value

Mackerel									
Dehydration time (hr) (%)	1	3	0	1	3	0	1	3	0
	2.1	3.5		2.3	3.8		1.6	3.7	
	2.2	3.5		1.8	3.3		1.5	3.4	
Frozen Storage at -20°C									
	5 days			30 days			-		
Fresh	14.8	16.5	18.2	18.1	18.5	17.2	19.7	21.9	20.3
	15.7	17.9	16.7	14.3	16.8	17.6	22.4	25.8	24.6
After Dehydration	18.3	21.9	-	21.0	21.7	-	22.3	23.1	-
	19.7	16.7	-	18.2	17.7	-	26.4	29.2	-
After Frozen Storage	17.6	19.5	15.2	18.2	19.6	18.4			
	15.2	14.5	14.1	15.7	16.3	14.1			
After Thawing	21.4	23.0	19.5	24.2	23.4	20.7			
	17.6	18.0	17.8	20.0	19.9	26.7			
Storage at 2°C 1 day	27.6	29.8	26.0	30.2	27.8	27.1	29.0	28.1	27.0
	27.4	23.1	24.9	27.3	25.4	29.0	33.0	34.8	28.9
2 days	33.7	34.8	33.6	38.6	33.4	34.2			
	31.9	28.5	30.1	33.4	33.0	34.6			
3 days							34.3	41.8	39.2
							42.9	42.1	38.6
5 days							41.4	45.4	47.6
							49.1	53.7	45.5
8 days	47.6	48.4	50.4	56.8	52.5	51.1			
	46.1	40.8	44.9	51.4	46.0	54.8			

ENTREPOSAGE A L'ETAT CONGELE DU POISSON ET DES PRODUITS DE LA PECHE AU JAPON ET NOUVELLE METHODE DE DESHYDRATATION-CONGELATION DU POISSON.

RESUME - On présente la pêche au Japon, la production de poisson congelé, les importations de poisson congelé et les durées d'entreposage recommandées pour les poissons et produits congelés de la pêche. On décrit brièvement une nouvelle méthode de déshydratation-congélation du poisson par élimination d'une petite proportion d'eau du produit avant la congélation.

SECTION IV

**AUTRES ASPECTS DE L'ENTREPOSAGE
DES PRODUITS CONGELÉS**

OTHER ASPECTS OF FROZEN STORAGE

RELIABLE REFRIGERATION ISN'T EXPENSIVE

A. H. BROWN

Star Refrigeration Ltd, Glasgow (United Kingdom)

1. INTRODUCTION

"Water entered the tank. A reaction began. The temperature rose. Stainless steel was corroded. A violent reaction. The safety valve burst. 2000 people died. Refrigeration was designed to play a key role in the safety procedure by slowing down the rate of reaction. But the refrigeration plant had not been working for over five months."

Refrigeration is designed to play a key role for chilled and frozen fish. Three days' discussion of their storage lives will be wasted if the refrigeration doesn't work. How does one ensure that one's refrigeration plant is reliable?

This paper discusses three themes of chilled and frozen storage — temperature control, mechanical reliability and long life — One consideration affects them all, the need for a generously sized cooler.

Why do people desire large engines in their motor cars? Perhaps they are enthusiasts; perhaps they want to go faster; perhaps they realise that they are going to abuse them. Large compressors also lead to abuse. Cars are for carrying people. Refrigeration is for making air cold. For that one needs the right cooler.

2. TEMPERATURE CONTROL

The advantages of lower temperatures on product quality are always being emphasised. Consider the effect of specifying a temperature of -30°C instead of -20°C .

Because of the lower temperature, the heat gains into the store may be 20% greater. To cope with that at the lower temperature, the compressors will require about twice the swept volume. The price of the refrigeration plant may increase by 30%. Yet refrigeration usually only represents about 20% of the total cost of a cold store installation. So the effect of lowering the specified temperature is an overall cost increase of 6%. That is less than 1% per degree.

Remember, however, that the refrigeration machinery will now take 60% more power. That is why so many stores are maintained at temperatures higher than those for which they were designed.

Temperature fluctuations usually relate to operational considerations. A store, designed only for storage, may be used as a freezer. Some plants are switched off when the engineer goes home. Others only run at times of reduced electricity tariffs. If temperature fluctuations arise from the refrigeration plant, then the control itself or the size of the cooler is likely to be responsible.

The temperature control (usually the bulb of a thermostat) must be positioned with care. The best position is often on the wall immediately behind or between the coolers. It is then in the stream of air returning to the coolers, representing the store temperature. It should be a little below the air coolers so that it is not affected by defrosts. It must not be near a door. It is often good practice to insulate it lightly so that it doesn't respond to transient effects.

I.I.F - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

There is no difficulty in obtaining temperature control within ± 1 deg C, so long as a good thermostat is used. Closer control is obtained by good design rather than extra expense.

Air temperature off a cooler fluctuates as plant stops and starts. The desirable air temperature drop across a cooler varies with product and air distribution. Below about 2 deg C, cooler fan power tends to be high. Above 4 deg C, the coolers are on the small side for plant reliability. The author suggests that a plant should normally run with an air temperature drop across its coolers of about $2\frac{1}{2}$ to 3 deg C.

To ensure even temperature throughout a store, some fans should run all the time. The economy of controlling all the fans on a cooler from a single starter is often self-defeating. Several large stores with multiple compressors are being run at times of high electricity tariffs with one compressor but all fans running. Fan power will then often account for over half the power being taken.

3. MECHANICAL RELIABILITY

The mechanical reliability of motor cars has improved continuously over our lifetimes. Over a shorter period, the same can be said of packaged refrigeration units for cold stores. Yet, just as the individual car may break down, so can any other mass-produced product. The best guide is reputation and experience.

The author's experience is in large custom-built low temperature cold stores. He wonders whether reliability has improved over the years. When the ceiling walls of a store were covered in pipe grids, there was no question of the adequacy of the cooler. It was the mechanical reliability of the compressor which was questioned. A standby would usually be installed.

Today, the quality of industrial refrigeration compressors, screw or reciprocating, has never been higher. If a plant is well designed, installed and maintained, little case can be made for a standby.

It is the fan circulated air cooler with its controls which is the weak link. They permit liquid refrigerant to spill over to the compressors to cause damage. They fail to defrost adequately and cause compressors to run outside their design conditions.

The essential for a reliable refrigeration plant is generously sized coolers, inspected and maintained regularly. This inspection implies the provision of easy and safe access to the coolers; they usually hang in a hostile environment.

Liquid refrigerant carry-over — the most frequent cause of compressor mechanical failure — can only be prevented by placing a generously sized liquid trap between the coolers and compressors. A pump circulation plant will already have such a trap, called perhaps a surge-drum, an accumulator or a low pressure receiver. It is the direct-expansion plant which needs one. Objection is often made that this trap will also trap any oil circulating with the refrigerant. It does; but that does not prevent the oil being returned to the compressors. Except on the smallest plants, such a liquid trap will add about 5% to its cost.

A frequent cause of liquid refrigerant carry-over, as well as a source of unreliability, is electric defrosting. There are so many hot-gas defrost systems available, even for small plants, that it is surprising that electric defrosting is still the norm. Not only do the electric elements themselves fail, not only do their high temperatures cause steaming, not only have their high electric loads even started fires, but also electric defrost running costs are high. The extra capital cost of hot-gas defrosting will usually be repaid in reduced running costs within a year.

After the cooler, it is simplicity which brings reliability. Why use a 2-stage system if a single-stage system will do. Resist the attraction of automatic capacity controls. Appreciate that good air distribution ensures steady air temperatures better than any sophisticated temperature monitoring and averaging device. Balance the advantages of cross-over connections against their risks. Don't specify ever more valves and controls.

As an example, compare the simple direct expansion system, which only has one isolating valve between cooler and compressor, with the typical central plant. It will have a control valve and isolating valve at each cooler, two isolating valves at its liquid trap and another at each compressor, a total of five. Pressure drops between cooler and compressor of over "5 deg C" have been measured on such systems. That is equivalent to 15% more power consumption on a -30°C store.

It isn't only the cost of that power. That extra power is being absorbed by the compressor to do the same amount of refrigeration. That much extra power cannot do reliability any good.

It is fashionable to want screw compressors instead of reciprocating. There may be advantages; but reliability isn't one of them. The author's experience is that if each is installed to the same standard, the one is as reliable as the other. Reciprocating compressors may need more frequent maintenance. Yet the cost of that maintenance over the long term will be no more.

British classification societies have reduced the frequency of mechanical inspections and no longer require spare parts to be held on site. Refrigeration plants can be reliable. Yet what happens in a breakdown?

Consider a 10,000 cu m, well insulated, cold store, holding 3,000 tons of fish at -30°C . If the doors are shut and the fans and lights switched off, the heat gain in summer might be 50 kW. After the temperature has risen a few degrees, any further rise will be restrained by the mass of low temperature product. 3,000 tons at 50 kW will only rise 0.8 deg C/day.

4. LONG LIFE

The oldest store with which the author is well acquainted is over 75 years old; it is still running on its original compressors. He does not suggest that as a target, or any target. The life of a store will depend on its usefulness. Refrigeration must serve that use.

The factor most likely to affect that service is the number of separate refrigeration circuits. A store might have ten factory-sealed units, "through-the roof" or "through-the-wall". Exposed to the elements, they may only have a limited life. Yet they can be replaced and uprated at any time to suit the changing needs of the store.

On the other hand, a custom-designed central plant could well out-live the store. Yet it would usually be more difficult and more costly to uprate it to suit changing needs.

For long life reliability, a compromise is suggested, two or three separate systems. When planning a new store, guard against professional advisers who will assume "the same as last time". The cost of separate systems imposed upon a plant intended for a central plant will often be prohibitive. Insulated pipe connections can cost more than compressors. If each system is installed next to its cooler, the cost can even be less than a central plant with its necessarily longer pipe connections.

Slow running reciprocating compressors are often specified. Historically, compressor reliability has improved as compressor speeds have increased. The important requirement is that any one compressor should run at the right speed for it and its application; that is often less than its maximum speed.

Just as the advantages of generously sized coolers have been emphasised, so a generously sized condenser will extend plant life. As the condensing temperature drops, so the compressor discharge temperature and power absorbed drop. These have a direct effect on its life.

The insulated pipe-lines should not be forgotten. Many plants have come to grief from a pierced vapour seal. It is too late to stem the flow once ice is observed. Replacement is then the only course. As with coolers, regular inspection and maintenance will be rewarded.

Simplicity has been recommended. So what about micro-processors? Although they are said to be more reliable than electro-mechanical controls, reliability from the better manufacturers cannot be criticised. The benefit of a micro-processor is not that it does things better but that it does so much more. The greatest of these extras is the link which it offers between the user and his maintenance service.

The maintenance engineer can now read the performance of each refrigeration plant from his own office and monitor it by computer. He can offer a comprehensive no-exclusions maintenance service. With the right quality of service, that could provide the ultimate in reliability. The micro-processor itself need already cost no more than conventional controls.

5. CONCLUSION

Reliability isn't expensive. To demand more, such as a lower temperature, costs more; but not a lot more.

Compressors may be at the heart of a plant. The controls may be its brain and the condenser its organs of rejection. But the cooler is its physique and its character, which achieves results and gives satisfaction. If the cooler is cared for, admired and caressed, the first step has been taken towards reliability.

As refrigeration plants become inherently more reliable, attention must turn to maintenance to secure that reliability.

UNE TECHNIQUE FRIGORIFIQUE FIABLE N'EST PAS COUTEUSE

RESUME : L'auteur examine trois aspects de la fiabilité des grandes installations frigorifiques pour entrepôts. 1. Les niveaux et les fluctuations de température dépendent des conditions prescrites et de la gestion de l'entrepôt. 2. L'auteur indique certains détails de la fiabilité mécanique. 3. Il propose que les installations frigorifiques ne soient pas conçues en vue d'une longue durée, mais en vue de leur adaptation à des changements futurs possibles des besoins d'entreposage.

En conclusion, il recommande que l'utilisateur d'entrepôt frigorifique s'intéresse aux frigorifères avant de s'intéresser aux compresseurs.

DISCUSSION

M. SANDERSON WALKER (U.K.) - Can I first comment on the paper by saying that it contains a large quantity of very practical advice. Secondly, can I ask whether the micro-processor systems described on the last page of the paper are now in commercial use ?

A.H. BROWN - Yes. There is a wide range of micro-processor systems available from the simple to the comprehensive. Some of these include a modem enabling the maintenance service described to be obtained.

PREDICTION OF STORAGE AND THAWING CONDITIONS BY
KINETIC PARAMETERS ON QUALITY CHANGES IN FISH MUSCLES

H. MIKI and J. NISHIMOTO
Faculty of Fisheries, Kagoshima University,
Kagoshima, Japan

1. INTRODUCTION

This study was based on the principle factors namely temperature histories and characteristic quality changes in fish after death, govern on the final quality of fishery products during distribution.

Furthermore, as affecting factor on food quality changes, were treated the different temperature of storage, pH, O_2 concentration, water activity, etc/1/2/. After the relation of those factors and quality changes became clear, the degree of changes on quality (which was based on temperature histories) could be predicted. However, the quality judgement on fish muscle are complex and difficult, and studies on the kinetics of quality changes are very few. Therefore, the subject of studies on mechanism of affect of time-temperature on quality changes in fish muscle was thought. On the other hand, the monitoring method on the integrator of time-temperature on food deterioration was devised /3/4/. Here, the authors had been investigated the rate of changes on freshness and discolouration on some species of fish, and as kinetic parameters the rate of quality changes with temperature dependence was obtained.

Accordingly, in the present paper, prediction on quality changes by using the kinetic parameters at fluctuating of temperature histories with time was tried. And then, the thawing condition for minimizing the deterioration was discussed by using the thawing curves of round frozen skipjack, and simulation by numerical calculation on freshness changes and discolouration of fish muscles.

2. EXPERIMENTAL

2.1 Fish Sample

Mackerel Scomber japonicus and skipjack Katsuwonus pelamis were obtained in fresh condition from a fish store, and were used to prepare the fish fillets for experimental materials. These fillets were kept at -70°C before preparation for the fluctuating tests of a storage temperature. For the thawing test, a round frozen skipjack (standard length 47 cm, and weight 2.3 kg) was used.

2.2 Fluctuating Test of Storage Temperature

At the beginning, sampling four pieces (ca. 3 g) of fish muscle from one fillet was done. These muscle pieces were analyzed to obtained the initial values of chemical indexes for freshness and discolouration. The other fillets of fish sample were wrapped individually with a polyethylene film, and were heated by standing in the room temperature and cooled by a freezer alternately two or three times, to cause fluctuating of storage temperature randomly. The temperature range of fluctuating was from -10 to 20°C in case of mackerel fillets, and was from -20 to 5°C in case of skipjack fillets.

2.3 Thawing Test

A round frozen skipjack was thawed by the still-air settled at 15°C after being equilibrated to a constant temperature of -25°C . The temperature changes during thawing were measured at the point of A(1/5 depth from a lateral line(l.l.)), B(2/5 depth from a dorsal fin (d.f.)), C(3/5 depth from l.l.), and D(4/5 depth from d.f.) in the middle of skipjack body respectively.

2.4 Measurement of Temperature

The thermocouples (C-C, 0.3 mm ϕ) were inserted into the internal fixed points of fish muscles before the tests. Thermocouples were connected to the automatic recorder of temperature (YEW Co.Ltd., IR-4036) and temperatures related with time were recorded.

2.5 Chemical Analysis

The K-value as a freshness-estimation index was analyzed by the column chromatography due to the method of KOBAYASHI et al [6]. The ion-exchanger used was Dowex ion Cl⁻ type with a mesh of 50-100. The metMb% for a discolouration-estimation index of red muscles was measured by BITO's method [7].

2.6 Prediction of Quality Changes from Temperature Histories

Provided that there is a fluctuating temperature during storage, it is possible to determine the change for a first-order reaction from the following equation [8].

2.303 log ($\frac{a}{a-x}$) = A $\int_0^t e^{-Ea/RT} dt$ (1)

where, a is initial amount, (a-x) is remaining amount at passed time t, A is frequency factor, R is gas constant, Ea is energy of activation, T is absolute temperature.

In the present experiment, it was tried to predict the changes of freshness and colour in the fish muscle during storage at fluctuating temperature from eq.(1). The rates of freshness-lowering and discolouration in fish muscles were investigated by using the chemical changes of (100 - K-value) and (100 - metMb%), respectively [5]. And then, as shown in tables I and II, their kinetic parameters (Ea, A) for exhibiting an ARRHENIUS-type dependence on temperature had been obtained.

TABLE I
Apparent activation energy(Ea) and frequency factor(A)
in the muscles of mackerel and sea bream .

Species	Temp. range	Ea (Kcal/mol)	A [h ⁻¹]
Mackerel	Above -2°C	16.231	2.269 x 10 ¹⁰
	-2 to -10°C	41.455	4.886 x 10 ³⁰
sea bream	Above -2°C	15.819	4.852 x 10 ⁹
	-2 to -10°C	33.615	7.178 x 10 ¹¹

TABLE II
Apparent activation energy (Ea) and frequency factor (A)
of the rate of freshness-lowering and discoloration in
the muscles of skipjack.

Rate constant	Temp. range	Ea [cal/mol]	A [h ⁻¹]
Freshness-lowering(k _f)	Above -2.0°C	1.399 x 10 ⁴	7.294 x 10 ⁸
	-2.0 to -10.0°C	5.092 x 10 ⁴	5.272 x 10 ³⁸
	Below -10.0°C	5.478 x 10 ⁴	8.024 x 10
Discoloration (k _c)	Above -2.0°C	2.521 x 10 ⁴	2.239 x 10 ¹⁸
	-2.0 to -5.0°C	9.054 x 10 ⁴	1.321 x 10 ⁷¹
	Below -5.0°C	1.829 x 10 ⁴	1.836 x 10 ¹²

Accordingly, the ratio of a quality change ($a/(a-x)$) to time could be determined by substituting the kinetic parameters into eq.(1). Provided the initial values of K-value(K_0) and metMbZ(metMbZ $_0$) are shown, these values (K_t and metMbZ $_t$) after t hours could be determined by eqs.(2) and (3), respectively, as follows :

$$a/(a-x) = (100 - K_0) / (100 - K_t) \quad (2)$$

$$= (100 - \text{metMbZ}_0) / (100 - \text{metMbZ}_t) \quad (3)$$

while, the integral of eq.(1) is calculated by the trapezoidal/9/ dividing the time into $dt = 1/6$ (h). These calculation were carried out by using the computer (FACOM 230, Kagoshima University).

3. RESULT AND DISCUSSION

3.1 Simulated Results and Experimental Data from Temperature Histories

3.1.1 Prediction of K-value Changes

Temperature histories of mackerel fillets after the fluctuating tests were indicated in fig. 1. From these temperature curves, the freshness changes of sample fillets after fluctuating temperature were predicted by using the eq.(1) as described in the 2.6 item. The results were shown in table III. The differences (ΔK) between calculated and experimental results were as much as 0.4 - 1.1 %. These differences showed that the two results were agreed approximately.

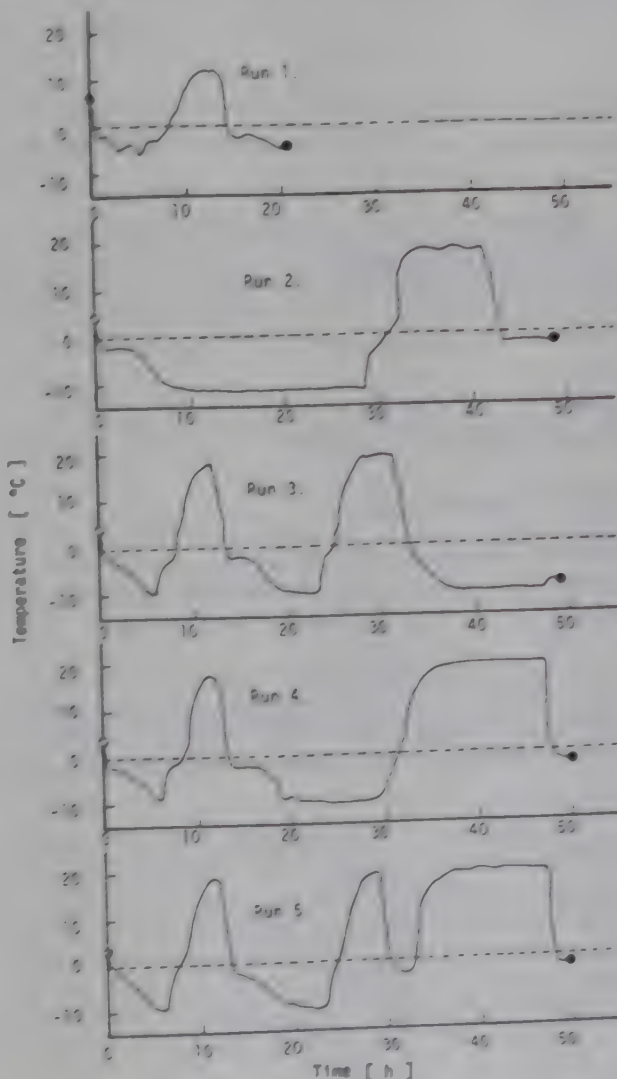


Fig. 1. Temperature histories for predicting changes of K value in mackerel fillet during fluctuating temperature storage.

- The initial measured point of K value.
- The final measured point of K value.

TABLE III

Comparison of the experimental results with the calculated results for predicting the K value after fluctuating temperature storage on mackerel muscles.

Run No.	Experimental		Calculated	ΔK	Run time [h]
	(1) Initial[%]	(2) Final[%]	(3) Final[%]	(3)-(2)[%]	
1	14.9	18.3	19.4	+1.1	20.7
2	5.7	20.3	21.1	+0.8	47.8
3	16.0	29.8	29.4	-0.4	48.8
4	16.4	37.0	37.8	+0.8	48.8
5	16.6	40.9	41.3	+0.4	48.8

Table IV indicated the simulated and calculated results of K-value changes of skipjack muscles after fluctuating temperature tests, in almost the same way as calculated in mackerel muscles. The changes of K-value ($K_t - K_o$) from the initial to the final time after the fluctuating temperature tests were small values, because of the fluctuating were under the low temperatures. However, the close agreement between the calculated and the experimental values were obtained.

TABLE IV

Comparison of the experimental results with the calculated results for K-value after fluctuating temperature storage on skipjack muscle.

Run No.	Experimental		Calculated	ΔK	Run time [h]
	(1) Initial [%]	(2) Final [%]	(3) Final [%]	(3)-(2) [%]	
1	5.89	6.58	6.91	-0.33	47.8
2	5.89	8.62	9.16	-0.54	48.7
3	5.89	10.80	10.54	+0.26	49.3

3.1.2 Prediction of Colour Changes

The colour changes of skipjack muscles after fluctuating temperatures were predicted in the same manner as prediction of K-value as above mentioned. These results were shown in table V.

TABLE V

Comparison of the experimental results with the calculated results for metMb% after fluctuating temperature storage on skipjack muscles.

Run No.	Experimental		Calculated	$\Delta \text{metMb\%}$	Run time [h]
	(1) Initial[%]	(2) Final[%]	(3) Final[%]	(3)-(2) [%]	
1	40.7	45.3	44.9	-0.4	30.8
2	35.7	42.3	40.5	-1.8	49.3
3	33.0	46.0	45.7	-0.3	48.2

The differences ($\Delta \text{metMb\%}$) between calculated and experimental results were small as much as -0.3 to -1.8 %.

The reason that the initial measured value (metMb₀) of discolouration was high in spite of fresh muscles condition might be resulted from the calibration for

reading the amount of metMb% as being pointed out by KAKUTA and UCHIYAMA^{*}.

3.2 Prediction of Quality Changes during Thawing

The thawing curves of a frozen skipjack was indicated in fig. 2. The change ratios of K-value and metMb% from the temperature histories during thawing which were expressed in eqs. (2) and (3) were calculated by eq. (1).

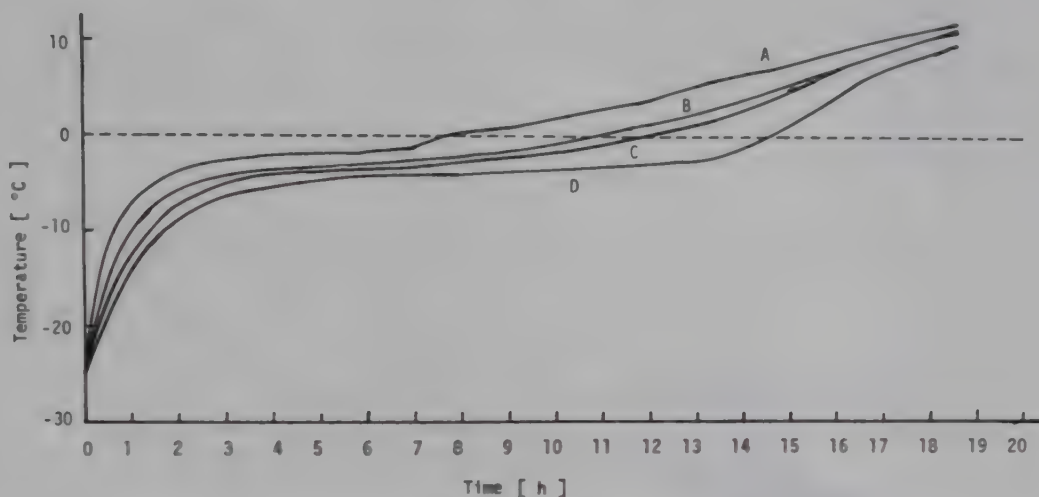


Fig. 2. Time-temperature curves in the round body of skipjack during still air-thawing at 15°C.

These change ratios of quality at various measured points were plotted in fig. 3 corresponding to the final temperature of thawing at the center portion of D point (described in the 2.5 item). These results indicated that the changes of metMb% were faster than that of K-value changes. This fact is evident on comparing the rates of K-value changes with that of metMb% changes as described in the previous paper/5/.

Assuming both of the initial values of K-value and metMb% on 10 %, it is considered that the calculated K-value on curves are not in excess of the 20 % line /10/ as the limitation for "SASHIMI" (raw fish muscle) at any final-temperatures. However, in case of discolouration it was predicted from fig. 3 that the metMb% curves in a surface layer might rise over the visual recognized line of discolouration (metMb% = 30 %)/11/, at the early time (i.e. near 0 °C of the final thawing-temperature).

In any case, it was estimated that the discolouration of skipjack muscles could be controlled within 1.1 times to the metMb% if the thawing operation was stopped near -3 to -5 °C or at the partial frozen stage as the final temperature. In this way, the hitherto recommended that the final thawing-temperature of -3 to -5 °C /12/ was theoretically supported to be optimum for the frozen fish which are thawed by heating from the outside. Accordingly, the present paper made it possible to determine the optimum condition for storage and thawing of fish evenly without deterioration of its freshness.

4. CONCLUSION

The prediction of changes on K-value and metMb% from temperature histories of fish muscle was exactly obtained numerically calculated. And then, by that methods of quality index, the quality changes on each part of round frozen skipjack (2.3 kg) during thawing by still-air (15 °C) was known and the result was calculated. As mentioned above, the availability of theoretical on partial thawing which was recommended from the experiences of the old techniques was proved.

The possibility for practical analysis of thawing curves on frozen fishery products has been done. Therefore, the quality changes in fish muscle on all process of freezing, storage, and thawing based on the relation of a time-temperature tolerance (i.e. kinetics of quality) could be simulated and monitored by a micro-computer.

^{*} Orally presented at the annual meeting of the Japan.Soc.Sci.Fish., on 1984, (Tokyo).

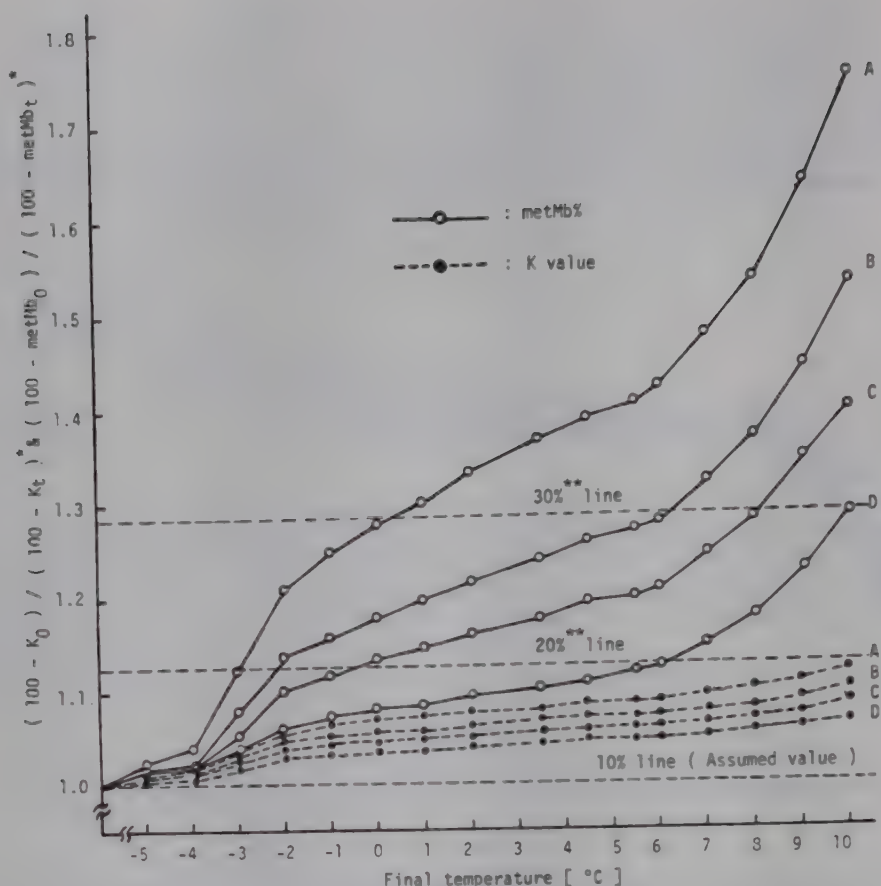


Fig. 3. Calculated ratios of quality changes (K values & metMb%) at different final temperatures of still air- thawing in the round body of skipjack.

* : Subscripts ; 0 = initial value , t = value at passed t hours.

** : Values of K_0 and metMb_t in case of 10% at initial time.

REFERENCES

1. Y.C. LEE, J.R.KIRK, C.L.BEDFORD and D.R.HELDMAN, J.Food Sci., 42, 640-644(1977).
2. T.P. LABUZA, J.Food Sci., 44, 1162-1168 (1979).
3. T. TOYOSHIMA, T. TOGAWA, A. KAMIYA, A. HAYAKAWA and T. KOBAYASHI, Refrigeration, 52, 1069-1078 (1977).
4. J.W. FARQUHAR, Int. J. Refrigeration, 5, 50-54 (1982).
5. H. MIKI and J. NISHIMOTO, Bull. Japan. Soc. Sci. Fish., 50, 281-285 (1984).
6. H. KOBAYASHI and H. UCHIYAMA, Bull. Tokai Reg. Fish. Res. Lab., No.61, 21-26 (1970), (in Japanese).
7. M.BITO and H.KIRIYAMA, Bull.Tokai Reg.Fish.Res.Lab.,No.75, 75-86(1973)(in Japan.)
8. S. CHARM, The Fundamentals of Food Engineering, AVI Publishing Co. Inc., Westport, Connecticut, 1983, pp. 510-522.
9. C.R. WYLLE, Calculus (translated by Y. TOMIHISA), Burein-tosyo-suppan Co. Ltd., Tokyo, 1971, pp. 29-30 (in Japanese).
10. H.UCHIYAMA and S.EHIRA, Bull.Japan.Soc.Sci.Fish.,36,977-992 (1970) (in Japanese).
11. M. BITO, Bull.Japan.Soc.Sci.Fish., 31, 534-539 (1965) (in Japanese).
12. T.TANAKA, New Food Industry, 11(6), 29 (in Japanese).

PREVISION DES CONDITIONS DE CONSERVATION ET DE DECONGELATION PAR LES PARAMETRES CINETIQUES DES CHANGEMENTS DE QUALITE DU MUSCLE DE POISSON

RESUME : La prévision des changements de couleur et de fraîcheur au cours de la conservation à des températures fluctuantes a été tentée à partir des paramètres cinétiques (énergie d'activation apparente; facteur de fréquence), obtenus par les auteurs. De plus, les changements de qualité du muscle de bonite congelée, au cours de la décongélation, ont été calculés par ordinateur. Les conditions optimales de décongélation en vue de minimiser la détérioration du muscle sont exposées et discutées.

En conclusion, les changements de qualités du muscle de poisson durant la conservation et la décongélation, basés sur la "Tolérance Temps-Température" (ou cinétique de qualité) peuvent être surveillés et simulés par micro-ordinateur.

DISCUSSION

S. KNØCHEL (Denmark) - As you have pointed out, K-value determination is basically a way of monitoring the enzymatic degradation of ATP related nucleotides. I would be interested to know whether you have been able to establish a temperature limit below which no reaction takes place and, if so, is this temperature limit then species - specific ?

H. MIKI - According to the data obtained in our previous paper (reference 151) the effects of temperature on the enzymatic reaction rate was quite low when the temperature was below -20°C . Accordingly it is hard to establish the limiting temperature at which no reaction occurs.

SIMULATION MODELLING OF FROZEN FOOD DISTRIBUTION
- RETAIL OPERATIONS AND THEIR EFFECT ON QUALITY

W.H.Earl, D.H.Allen,
Technological Economics Research Unit,
University of Stirling. United Kingdom

L.Kindleysides,
Unilever Research, Colworth Laboratory (United Kingdom)

. Introduction.

This paper describes progress on work carried out as part of a research project entitled "Systems Modelling of Refrigerated Food Distribution", being undertaken at the University of Stirling with the support of Unilever Research and Birds-Eye Walls Ltd.

The logistics of distribution systems in general have been studied and modelled many times in the past. Commercial software packages have been available for some time which can assist management in optimising the day to day operations of the distribution chain by such techniques as linear programming. However, when consideration is given to the products which actually pass through the distribution system some wider issues must be taken into account. This is clearly so when dealing with food products, particularly for refrigerated products where temperature management becomes an important issue. The concept of time/temperature tolerance for frozen products is well known [1], and highlights the interactions between time, temperature and quality. The inherent quality of frozen foods is high but poor storage conditions can result in quality loss. The retail outlet, particularly the open top display cabinet, has long been regarded as the weak link in the distribution chain [2].

In the wider sense the frozen food distribution system contains many interacting parameters which control what is a complex operation eg. sales demand, different product types, stock rotation procedures, temperature, quality factors. This work is aimed at modelling these complex interactions so that the effects of proposed changes/improvements can be tested, the possible benefits of new approaches to distribution analysed etc. The models are not intended to be used for operational control in practical systems, but are meant to be used as a research tool.

Methodology.

When attempting to predict the results of any proposed change to an existing distribution chain, direct experimentation on the real system is not feasible for the following reasons:

- (a) It would disrupt company operations;
- (b) Due to people being an integral part of the system, results may be affected by the 'Hawthorne Effect' - ie. the fact that people are being observed may cause them to modify their behaviour;
- (c) It would be very difficult to reproduce the same operating conditions for each run of the experiment;
- (d) It would be extremely costly to obtain statistically significant sample sizes from the real system;
- (e) It would not be possible to modify the existing system to any great extent to explore completely different alternatives.

Modelling the real system by means of computer simulation offers an alternative methodology which can allow all of the above problems to be overcome. Simulation models are input-output models, ie. they yield the output of the system when given the inputs to its interacting subsystems. They are incapable of generating an optimum solution by their own means, but can serve as a tool for the analysis of the behaviour of a system under conditions specified by the experimenter. Simulation modelling can then prove useful both for understanding the behaviour of systems, and for evaluating various strategies for the operation of systems.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

Looking at the various stages in the distribution chain, it is clear that the area where least information on the system behaviour is available is in the region of the retail outlet. While manufacturers are able to monitor quite closely the age and temperature of products within their own distribution chain, once the products are delivered to the retail outlet very little control is possible over subsequent handling and storage conditions. Until recently no large scale survey had been carried out in the U.K. to ascertain the storage conditions or residence times actually encountered in the retail outlet. As a starting point for modelling the entire distribution chain therefore it was decided to develop a computer simulation model of the handling and storage of frozen food as it passes through the backup stores and display cabinets of the retail outlet.

The Model.

1. Residence Times.

The first stage in developing a simulation model of the retail outlet has been to produce a computer program which is able to simulate the physical manipulation of the product as packets are moved in and out of the display cabinet. This was achieved by representing the layout of the display cabinet as a three dimensional array set up within the memory of the computer. Each element within this array then represents the location of one packet in the display cabinet. Each element of the array contains a number which is the residence time of the packet in that particular location. If a location is empty then a zero digit is held in the corresponding array element. This arrangement has proven entirely satisfactory for those products packed in boxes which can be stacked in the cabinet. Products in loose-fill packs, such as bags of frozen vegetables, can also easily be approximated to some form of regular stack.

Having established a model which can simulate the packs being placed into and removed from the cabinet, various options are required to make the inputs and outputs realistic. Inputs to the model, representing stock additions to the display cabinet, may be made under both First-In First-Out (FIFO), or Last-In First-Out (LIFO) stock rotation regimes. The model allows the user to select a case size, which is then taken to be the minimum re-order quantity, and only multiples of this figure can be added to the cabinet.

To simulate LIFO stock additions the empty locations at the top of the array are counted. If there are sufficient to hold at least one case of product, the appropriate number of locations are filled with the age of the product as it enters the cabinet. To simulate FIFO the new packs must be placed on the bottom layers and the data corresponding to the old packs replaced on top. When FIFO stock rotation is used the model allows three options for adding back the old stock; (a) it can be replaced in stacks in the same order it was removed; (b) it can be replaced at random; or (c) stock can be sorted with the oldest packs at the top. With the model permitting any combination of FIFO and LIFO to be used, it can deal with all of the possible operating policies in a real system.

The next part of the model represents the customer removing packs from the cabinet. For the purposes of this model it has been assumed that packs are only removed from the uppermost layer of product in the cabinet. It may be argued that some customers 'rummage' through the stock and select packs from elsewhere in the cabinet, but in the absence of detailed data being available on this point the above assumption is believed to be as realistic as any other option would be. The demand figures for the simulation, giving the number of packs to be removed each day must be input as part of the operating conditions at the start of the run.

An additional feature added during this physical manipulation phase was to provide the facility to simulate a regular cleaning cycle for the cabinet. After the cabinet has been emptied for cleaning the stock may be replaced by any of the three methods described under the FIFO sequence above.

Having developed the model this far, various operating policies have been experimented with to investigate their effect on residence times. This has revealed that when the system is run under a LIFO stock rotation regime the vast majority of packs pass through the cabinet fairly quickly. The remaining packs are however likely to become buried beneath newer stock, and thus could experience very long

residence times indeed. At first sight it appeared that these residence times were extremely unlikely to be allowed to occur in a real system, and so some supporting evidence was sought.

A general survey of frozen food distribution was undertaken in 1982/83 as a joint effort by staff from the Torry Research Station, the M.A.F.F. Food Science Division, and the Campden Food Preservation Research Association. The results of this survey were communicated to representatives of those companies and organisations who co-operated in the analysis of the data collected at a seminar held in May 1984. The residence times derived in the survey were based on the date on which products were placed into their final retail packaging, thus giving the cumulative time within the whole distribution chain rather than just in the retail outlet. Even so, residence times of up to 840 days were recorded, giving ample indication that this magnitude of residence time can actually occur within a real system.

2. Temperature.

The next phase in the development of the model was to incorporate temperature data into the model in order that the time-temperature tolerance (TTT) factors could be assessed to predict quality loss in the products. Analysis of the temperature data from the M.A.F.F. survey and other published work on the subject revealed that no one temperature distribution could represent the results of all the work conducted. It was decided therefore to allow the user of the model to set whatever operating temperatures were required for each simulation. The temperature may be set as being constant for the whole cabinet, a different but constant temperature for each layer, or a mean and standard deviation for each layer. In addition, a default value approximating the results from the M.A.F.F. survey may be used [3]. The model uses actual product temperatures rather than the air temperature within the display cabinet.

3. Product Quality.

The final phase to be included in the model was that data which allows product quality changes to be calculated. This is a very difficult concept to handle in a computer model. The model predictions can only be as good as the quality factors used. The tolerance element of the TTT concept can be subdivided into the product, processing, and packaging (PPP) factors. Each of these factors can vary enormously, and to produce a model with built in TTT data for even a small selection of the possible alternatives would be largely impractical, and it was therefore decided to construct the system so that users would input their own quality data. A further problem with assessing the loss of quality in food products is that the comprising quality factors are rarely quantifiable or measurable values. This results in the quality assessment of the food often being based solely on the subjective analysis of human taste panel evaluations. Although experienced tasters are often used, and the industry is satisfied that this is the best test available, the descriptive factors used in this form of assessment makes computer modelling of the process extremely difficult.

Both of these problems have been overcome by basing the model on shelf life data rather than on individual quality parameters. By allowing the user to input a segmented shelf life curve the model can cope with whatever form of quality assessment the experimenter cares to employ.

The shelf-life data is input to the model in the following way. The user first inputs the maximum temperature the product can endure without being spoilt almost immediately due to thawing etc. The model then has a built in counter to check how many packs exceed this temperature over the course of the simulation. If a maximum temperature is not required for the product then some arbitrary figure representing the highest temperature expected to be encountered should be used. From this maximum temperature the user then plots a series of points of shelf-life against storage temperature, down to a temperature of -40 C. The model connects these points by a series of Arrhenius plots and gives the user the predicted shelf life at the mid-point of each range. If the accuracy achieved is unsatisfactory a smaller temperature range must be used for each segment of the curve until the user finds the overall shelf-life plot acceptable.

The calculations used to construct the Arrhenius plots are based on the assumption that the quality deterioration is being caused by a zero order reaction. Depending on the reaction being considered its order can in fact lie anywhere between zero and two. Investigations have shown that several modes of quality deterioration do follow the rules of zero order kinetics [4]. Of these, both enzymatic degradation and lipid oxidation are applicable to the storage of frozen foods. Other modes of deterioration have been approximated to follow half, first or second order reaction series. However, when complex foods are considered, as with most frozen foods, the data best fits to zero order kinetics.

For the purposes of this model the quality of the product is assumed to be 100% at the time it enters the retail outlet. Thus the quality loss will be 0% at this point, building up to 100% when the product reaches the end of whatever measure of storage life is used. Typically this could be the Practical Storage Life; ie. the time at which a change in quality would become noticeable to a consumer.

The quality loss calculations are updated once for each day of computer simulation. At the start of each day the temperature of every pack in the cabinet is sampled, and this temperature is assumed to have been the average storage temperature over the preceding 24 hours. Comparison of this temperature with the shelf-life data then allows the percentage quality loss as a result of that storage to be calculated. This methodology works well as long as the assumption holds that a change in temperature does not in itself cause any deterioration in quality, ie. that 3 months at -18 C followed by 3 months at -12 C will result in the same quality loss as a pack stored for 6 months with the temperature fluctuating between -18 C and -12 C on alternate days. In this somewhat extreme example it is doubtful whether the assumption would hold, but for the limited number of large temperature changes likely to be experienced in a real distribution chain, and in the absence of any comprehensive data being available on the effects of the actual change in temperature, the assumption is the most reasonable which could be found. Following on from this it is also assumed that no quality loss results directly from the action of removing and replacing the old stock during FIFO stock additions, or during the cleaning cycle. Finally, using the same assumptions and procedures outlined above, the model has been extended to include a back-up coldstore. This allows the model to simulate more accurately the large supermarket environment where such storage is often available.

Results from the Model

The model in its present form is able to simulate stock movement through the back-up stores and display cabinets of the retail outlet. Output from the model consists of two columns of figures, one containing the residence time, and the other the percentage quality loss, of each pack leaving the system. Interpretation of these results will allow the user to identify the key operating variables within the system, and indicate how great the effects of any changes could be on product quality.

One such area of key importance has been identified as being the method of stock replenishment. For the purpose of demonstrating this effect, some practical storage life data for a model product has been used to produce the results shown in figure 2; ie. 20 days at -5 C; 80 days at -15 C; 160 days at -25 C; and 300 days at -40 C.

The series of histograms in figure 1 show the results of simulation runs identical in all aspects apart from the stock rotation policy. It can be clearly seen from these results that the stock rotation employed can produce very different distributions for the residence times and quality losses of the packs passing through the system. FIFO (reference figures 1a and 1c), the recommended procedure, ensures that all products are well within their practical storage life at the time of purchase. The adoption of a LIFO policy however (figures 1b and 1d), produces much longer tails in both distributions. Thus a significant number of packs approach their practical storage life limit and, in the particular example shown in 2d, 0.8% exceed this limit.

FIGURE 1a: Histogram showing residence times against percentage of packs when FIFO used

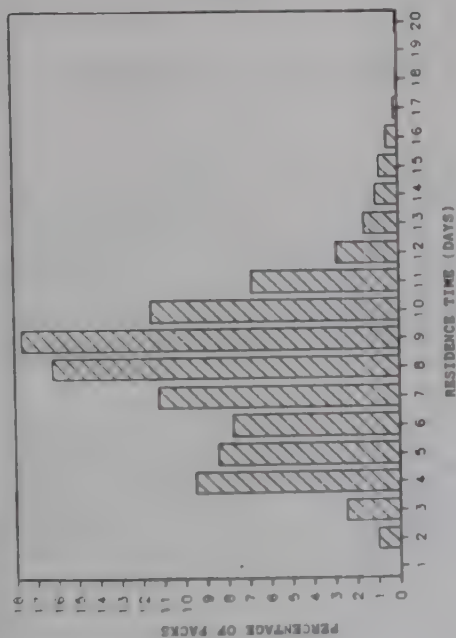


FIGURE 1c: Histogram showing percentage loss of P.S.L. against percentage of packs when FIFO used

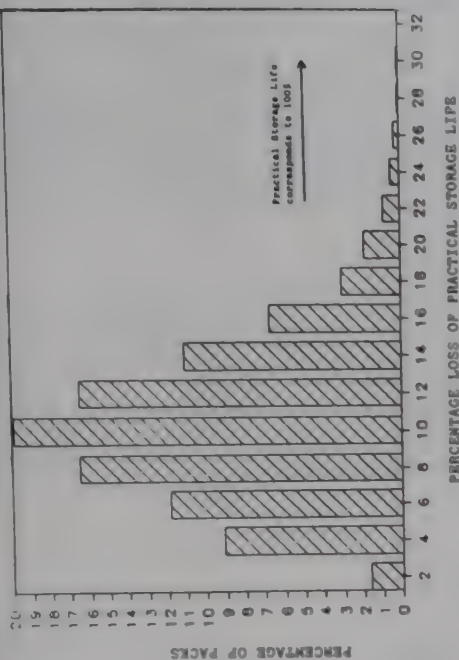


FIGURE 1b: Histogram showing residence times against percentage of packs when LIFO used

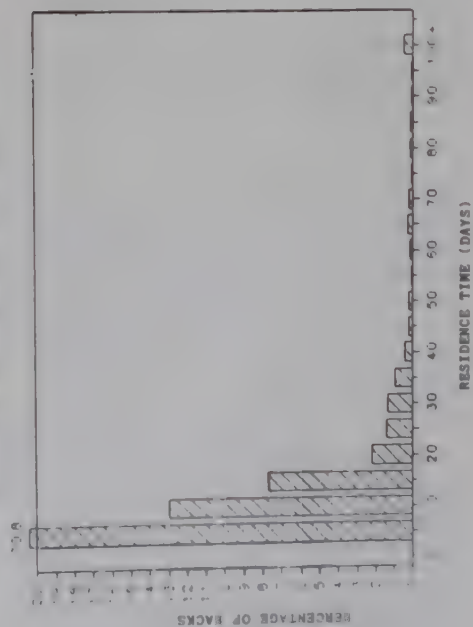
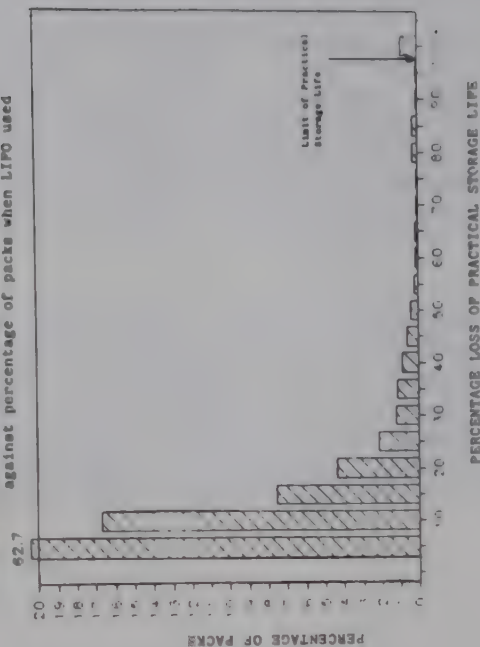


FIGURE 1d: Histogram showing percentage loss of P.S.L. against percentage of packs when LIFO used



There are however several points which should be remembered when dealing with the results from a simulation model. Firstly, a simulation model may appear to be satisfactory when in fact it does not properly reflect the real world situation being modelled. Great care must be taken in the construction of the model if problems are to be resolved correctly, and the model must be validated against the real system. Secondly, simulation is imprecise by its very nature of approximating the behaviour of a real system. The degree of this imprecision cannot easily be measured, but may be partly overcome by means of a sensitivity analysis of the model to changing parameter values. Finally, simulation results are usually numerical, and can be given to any number of decimal places the experimenter wishes. It is important to remember this fact and not to attribute to the numbers a greater degree of validity than is justified. Having recognised these possible downfalls however, with proper care simulation modelling can prove a highly useful tool in the investigation of a system's behaviour where the real live operation is not easily open to experimentation.

Validation of the model and a full sensitivity analysis is currently being carried out. Further work is also proposed to investigate the conditions experienced by products in other areas of the retail outlet, eg. the handling of the bulk order as it is delivered to the store. The inclusion of these factors into the model should produce a comprehensive simulation of the handling and storage of frozen foods throughout their stay in the retail outlet. The next stage of the work is to consider linking the model of the retail outlet into an integrated model of the whole distribution system.

References

1. Van Arsdel, W.B.; Copley, M.J.; Olson, R.L. (1969)
Quality and Stability of Frozen Food. Wiley Interscience, New York.
2. Lorentzen, G. (1971), Present Status of the Freezer Chain.
Scandinavian Refrigeration, Aug-Sept 1971 pp.5-11.
3. Potter, D.P. and Wignall, J. (1984)
The Time and Temperature of Frozen Food in Distribution,
Torry Document No. 1865.
4. Labuza, T.P. (1982) Shelf-life dating of foods. Food and Nutrition Press.

LA REPRESENTATION PAR MODELES DE SIMULATION DES OPERATIONS DE DISTRIBUTION ET DE DETAIL DES PRODUITS ALIMENTAIRES CONGELES.

RESUME : La manutention et le stockage des produits congelés dans le commerce de détail peuvent être schématisés par un système complexe de plusieurs variables dépendantes. Il est de la plus haute importance pour les fabricants de produits et pour les commerçants de détail que la qualité de ces produits soit conservée. La simulation par ordinateur offre une méthode pratique d'étudier les interactions et leurs effets sur la qualité. Pour la première étape de la simulation du système de distribution d'aliments congelés, on a mis au point un modèle qui représente les conditions du point de vente.

Le modèle simule les périodes de séjour des produits dans la chambre froide et dans les meubles de vente. On a établi un rapport temps-température pour chaque paquet et on l'a utilisé, avec des données sur la durée de vie des produits entreposés, pour prévoir la perte de qualité des produits qui en résulte. L'utilisateur peut définir les conditions de contrôle requises, comme le mode de rotation des stocks, la fréquence des livraisons, les niveaux de demande, les températures opérationnelles, les caractéristiques de la qualité des produits, etc. Le modèle donne une sortie sous forme de période de séjour et sous forme de répartition des pertes de qualité.

Les résultats sont présentés; ils montrent les effets de la modification des variables clés, et ils indiquent les domaines d'instabilité particulière pour la qualité des produits. On expose dans ses lignes générales la mise au point prévue de ce modèle.

DISCUSSION

R.M. GORDON (Guyana) - It is likely that a retailer may wish to monitor more than one product at the same time. Can the model be adapted to take account of the simultaneous movement of more than one product and in particular, the marketing factors which would influence the movement of competing products such as beef, lamb and fish ?

W.H. EARL - The model is restricted to a single product due to computational time restraints for each simulation. The model can take into account the marketing factors mentioned by manipulation of the demand profile of the input data. It is not, however, designed primarily as a marketing model, but as a quality assurance model.

ACCEPTABILITY OF FROZEN STORED CONSUMER FISH PRODUCTS

I.M. MACKIE, P. HOWGATE, A. CRAIG, W.M. LAIRD and A.H. RITCHIE
Ministry of Agriculture, Fisheries and Food, Torry Research Station,
Aberdeen (United Kingdom)

1. INTRODUCTION

With the wide acceptance of the value to the consumer of date stamping of perishable foods, it is likely that this open form of labelling will be incorporated increasingly into national legislation. Within the EEC, the directive on food labelling /1/ seeks to impose a uniform approach to date stamping of prepacked foods, including frozen foods, as part of its policy of harmonisation of legislation. The implication in such a directive is that internationally agreed criteria for the shelf life of frozen fish products should be used.

Unfortunately, as references to publications on shelf life show /2/, no such state of affairs exists largely because there has been less urgency for foods which present no health hazard. Criteria for acceptability of frozen products are also more difficult to recognise than those for perishable non-frozen foods as they do not have distinct off odours and flavours associated with bacterial spoilage. For the latter foods, objective criteria such as maximum permitted bacterial loads can be used to measure shelf life and, as they generally correlate with off-flavour development, shelf life assessment is relatively straightforward.

For frozen foods, and particularly for fish, shelf lives are usually determined from measurements made by trained assessors /3/ and a judgement is then made on the likely acceptability of the product to a typical consumer. It would appear that few of the published storage lives of frozen fish have been based on actual assessments by consumers /2/. Because of this lack of published information on consumer acceptability of frozen stored fish products, we decided to extend our assessments beyond those of our own panels at Torry Research Station (TRS) with the intention of obtaining opinions more representative of the general population of consumers.

After consultation with the UK Association of Frozen Food Producers we set up a storage trial of those fish products with high value retail sales in the UK - breaded fillets of cod, glazed fillets of cod, fish fingers of cod fillets and fish fingers of cod V-cut mince. Through the cooperation of 9 Colleges of Home Economics throughout the country we have been able to obtain assessments of these type of products by 350-400 individuals and we have related the data to our own hedonic and trained panel ratings.

The results presented are part of a larger storage trial of retail fish products, the results of which will be published elsewhere.

2. MATERIALS AND METHODS

Preparation of 'retail' fish products

Four products of cod (*Gadus morhua*), namely breaded fillets, glazed fillets, fillet fingers and mince fingers, were prepared.

Cod of TPS freshness score 8 were obtained from Aberdeen fish market by a local processor and made into primary products - individual skin-on fillets, blocks of fillets and blocks of V-cut mince. The fillets were frozen in TRS with one half being glazed immediately after freezing and the other half transferred to a local factory for breading. The fillets were packed loosely in plastic bags for storage. The blocks of fillets and of mince were processed in the same factory into breaded fish fingers and made up in retail packs of 10 in waxed cartons. The products were transferred with minimum delay to cold storage at -6°, -12°, -18°, -24° and -30°C. At intervals throughout one year samples which had been stored at -18°C were removed for assessment by TRS and the Colleges of Home Economics. Samples stored at the other temperatures were also examined but only by the TRS panels, and by chemical tests at TRS.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

Sensory Testing

Samples for hedonic rating were cooked from the frozen state by frying in oil. The uncoated fillets were first dipped in beaten egg then coated with breadcrumbs. Samples for testing by the expert panel at TRS were thawed, or partially thawed, and the coating scraped off before cooking by steaming in casseroles over boiling water.

Samples were rated for liking at the colleges (350-400 assessors) and by the non-expert panel at TRS (42 assessors) on the 9-point hedonic scale of Peryam and Pilgrim /4/. On this scale 1 = dislike extremely, 9 = like extremely and 5 = neither like nor dislike. The expert panel scored for cold storage flavour (CSF) on an intensity scale ranging from 0 = absent, to 6 = extremely strong. Toughness was scored on a bi-polar intensity scale anchored at the ends by 1 = very tender and 10 = very tough.

Dimethylamine (DMA) determination

Dimethylamine was determined by gas chromatography of the hydrochloride /5/.

3. RESULTS AND DISCUSSION

The hedonic ratings were pooled over the 9 colleges to give data from 350-400 assessors for each product/storage combination. All ratings from 1 through 9 were represented in the data though very few ratings of 1 were given. The initial modal value was 7 for all treatments except fillet fish fingers for which it was 8. About 80% of ratings were in the range 4 to 8, but the distributions for all samples were negatively skewed. Because of this marked skewness the median has been used as the measure of central tendency rather than the mean and differences between samples have been tested for significance by non-parametric methods, the Kolmogorov-Smirnov and the Wilcoxon pair comparison tests. In calculating the median the underlying property being measured is assumed to be continuous, even though the rating scale takes discrete values, and the median is calculated as a real number.

Median values given by the college population for the treatments are shown in Fig. 1a. There are two conclusions to be drawn immediately from the Figure: there is a clear differentiation in liking among the products; with the exception of glazed fillets there is little change in liking over 12 months of storage.

The product most liked by the college population was fish fingers prepared from fillets but fish fingers prepared from fillet mince, even though appearing the same externally, were least liked. It can therefore be concluded that the assessors were responding to differences in the organoleptic properties of the core material and that differences were not swamped by the coating. Fillets stored in the glazed form were initially liked to about the same extent as breaded fillets, though they became less liked during the period of storage. For three of the products, both types of fish fingers and the breaded fillet, the median ratings after 12 months were close to, and not significantly different from, the initial values. The medians for the glazed fillets however show a trend downwards during storage.

The median score is the value such that 50% of the ratings were above and 50% below it. It does not directly give a measure of the proportion of people who like the product and a change in the median value does not directly translate into a change in proportion of people who like the product. A better index for shelf life studies is perhaps the % of people expressing a liking for the product. Ratings of 6 and more indicate directly some degree of liking. A rating of 5 is the neither like nor dislike range but if liking is continuous it can be assumed that approximately half those giving this rating like the product, even to a vanishingly small extent, and half dislike it. The % liking is then the % giving ratings of 6 or above plus half the %age giving 5. The values for the college population are shown in Fig. 1b.

The general picture shown by the % liking values is the same as that shown by the medians but the actual values are of interest. Only somewhere between 72% and 82% of the assessors showed some degree of liking for the initial samples. A discussion of why this value is not 100% is outside the scope of this paper but it must be borne in mind in the context of discussing criteria for measuring the end of shelf life since it is often assumed that the initial material is completely acceptable. If the end point is taken as some fixed value of % liking then even if various products change during storage at the same rates their shelf lives will be different

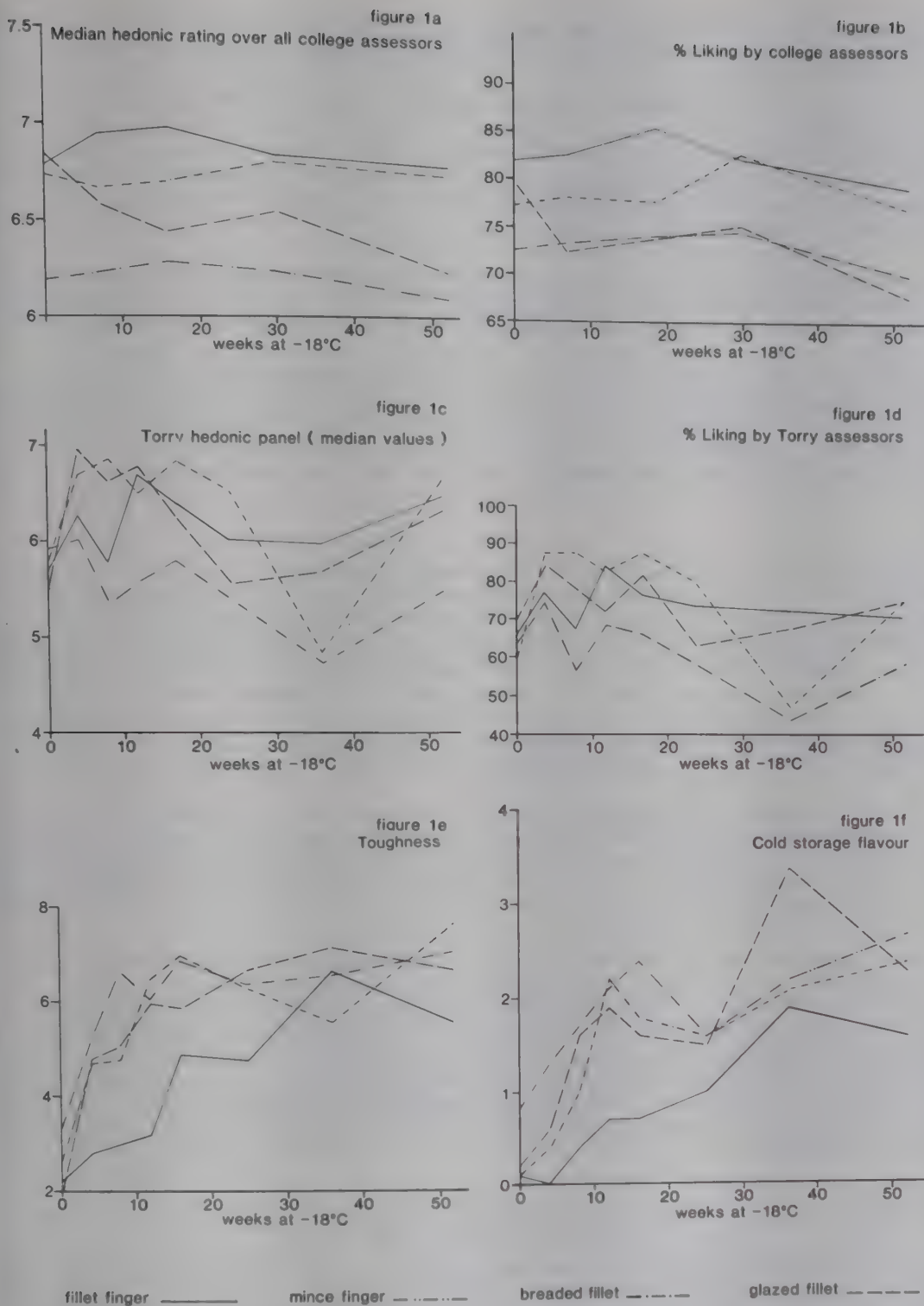


Fig. 1 - Sensory panel results

if the initial values differ. For example, if the end point is fixed at 75% liking then fish fingers prepared from mince have a zero - or even negative - shelf life. This is clearly not the case in practice since mince fish fingers are popular. A marketing manager, knowing the typical sales of a product, may be more interested in measuring the effect of storage by a change in liking as an indication of potential loss in sales. By this criterion only the uncoated fillets showed a marked decrease in liking during storage.

The equivalent data from the TRS panel are shown in Figs 1c and 1d. The values are obtained for panels of only 42 assessors and are considerably less

precise than those from the college panels. As a result the data are more erratic than in the case of the college data but similar patterns emerge.

Over the period of storage the TRS assessors most liked the breaded fillets, though not appreciably more than the glazed fillets or the fish fingers made from fillets; over the whole storage period the mince fish fingers were least liked. The Torry panel also assessed the products stored at other temperatures and those data confirmed that the mince fish fingers were least liked with the other products being liked to about the same extent.

A marked feature of these products is the very low median scores given to the starting material by the TRS panel; values for similar products, including those from mince, from many storage and acceptability trials conducted at Torry over the last 5 years or so have been above 6.5. We had not previously met values for nominally good quality products as low as those met in the trial described here. The Torry assessors were not formally asked to record their observations or opinions about the product other than by allocating a rating, but it was notable that many assessors after appraising these initial products commented in writing on the score sheet or verbally to the panel manager that they were very soft and mushy. The initially very soft texture is confirmed by the expert panel scores to be described later.

Figure 1c shows that the median scores were higher after 4 weeks of storage. We believe this is a real effect and not due to variability in the panel since medians for the products stored at -6°C , -12°C and -24°C also showed the increase at the first sampling points of 1, 4 and 12 weeks respectively except for fish fingers from mince held at -24°C . (The medians of samples stored at -30°C showed almost no change and retained the low zero time values over the 12 months storage period.) The mince fish fingers show the smallest increase (in fact the value at 4 weeks is not significantly different from that at zero time) and the increases for this product at -6°C and -12°C already referred to were also very small.

It is not easy to draw conclusions about changes from the results shown in Fig. 1c but there does appear to be a tendency for the medians to decrease after the early increase even though overall there is no marked decrease. The value for coated fillets sampled at 36 weeks is very low. We have no explanation for this though we believe it is abnormal in some way.

The values for the proportion liking by the TRS panel are shown in Fig. 1d. The fish fingers prepared from mince is the least popular with little difference among the other products. The proportions liking the products at the beginning are very low at less than 70%; white fish products like these typically receive over 80% liking by the TRS panel. The proportion liking increases markedly over the first 4 weeks of storage and apart from mince fish fingers increases a little more during further storage. Again, there is no clear tendency for the proportion liking to decrease overall during storage (not including the low value for coated fillets sampled at 36 weeks).

The results of the expert assessments of specific attributes are shown in Fig. 1e, toughness, and Fig. 1f, cold storage flavour (CSF).

The toughness scores at zero time are very low; in fact they are among the lowest ever given by the panel to samples of cod fillet or mince. Unfrozen cod fillets usually have mean scores in the range 4 to 6 on this toughness scale with minces, as here, getting a score about one unit higher. The products toughened during storage with most of the increase in score for the 2 fillet products and the mince fingers occurring within the first 3 months. The fingers prepared from fillets toughened more slowly than the other products at -18°C , as shown in Fig. 1e, and also at the other storage temperatures.

Figure 1f shows that fish fingers prepared from fillet have the lowest rate of production of CSF (this is also true at the other storage temperatures). Theoretically, the samples at the start of storage should have a score of zero but in practice they usually have a small positive value since assessors do not know their origin and some may detect, or think they detect, cold storage flavour in a sample and hence give a score to it. These positively biased scores cannot be balanced by negatively biased ones since scores below 0 are not permitted and the panel mean takes a positive value. The starting value for the fingers made from mince is higher

than this error and truly reflects the typical flavour of mince from white fish which is very similar to the cold storage flavour /6/. The subsequent rate of production of cold storage flavour at -18°C is similar to that in fingers prepared from fillet, as shown in Fig. 1f, and also at the other storage temperatures.

This trial does not give a clear-cut estimate of the shelf lives of the products studied and in fact illustrates many of the problems of both defining and measuring storage lives. The results from the college assessments suggest that the acceptability of 3 of the products does not decrease over 12 months storage at -18°C . This is unlikely to be the result of insensitivity of the assessors as a group to differences in organoleptic properties of fish since they consistently differentiate in liking among the products. Also the median scores and % liking of the glazed fillet show decreases, though small, over the period.

If change in liking is used as the criterion for determining shelf life then perhaps only the glazed fillet product can be allocated a value on the results from this study. For example, if a decrease of 0.5 hedonic rating units or a decrease of proportion liking of 10%-age units are used as criteria then the shelf life is about 9 months at -18°C for the glazed fillets and some value well over 12 months for the others.

It is surprising that the hedonic panels did not respond to changes in organoleptic properties during storage. There is no doubt that there were changes as the scores for toughness and CSF given by the expert panel show. Though these changes are quite large they do not seem to have influenced the hedonic ratings. Conversely the hedonic ratings for glazed fillets and for breaded fillets are consistently different throughout the storage period though CSF and toughness scores are essentially the same for the 2 products.

In contrast, the TRS hedonic panel does appear to respond to changes, and particularly to changes in texture, during frozen storage. The increase in liking over the first few weeks of storage is associated with the increase in toughness scores given by the expert panel. During this time the small increase in CSF is probably not having an influence but the trend for liking to decrease during later periods of storage is associated with increasing intensities of CSF. However the degree of liking at 52 weeks of storage is similar to that at 0 weeks. Connell and Howgate /7/ reported a trial which suggested that flavour influenced hedonic rating more than texture, but in those experiments the fillets were not coated before frying and the toughness was in the normal range.

The expert panel data could be used to establish a limit to storage life if there were some relationship between CSF, toughness and consumer acceptability. A cold flavour score of between 2, slight, and 3, moderate, intensity should be detectable to most people but CSF does not appear to have influenced the college assessors. It is possible that the method of preparation and cooking of the samples for the hedonic tasting masks CSF so that it is not noticeable to non-expert assessors. Toughness which is often assumed to affect acceptability of frozen stored fish did not reach very high scores.

The data for the dimethylamine content of the four products show steady increases with time of storage (Fig. 2a). Dimethylamine is produced enzymatically during frozen storage by the action of trimethylamine oxide (TMAO) demethylase and its concentration in flesh is a useful index of frozen storage deterioration. It would appear that its rate of formation in the fillet finger is somewhat slower than in the other three products, in line with observations on toughness and CSF formation.

A comparison of DMA production in the mince fingers at the other temperatures of storage (Fig. 2b) shows the dependence of rate of formation on storage temperature. Values in excess of 5 mg DMA N/100 g, usually indicative of extensive frozen storage deterioration, are obtained after 36 weeks at -18°C or 10 weeks at -12°C . That is, there is nothing untoward in the rates of deterioration as assessed by chemical criteria.

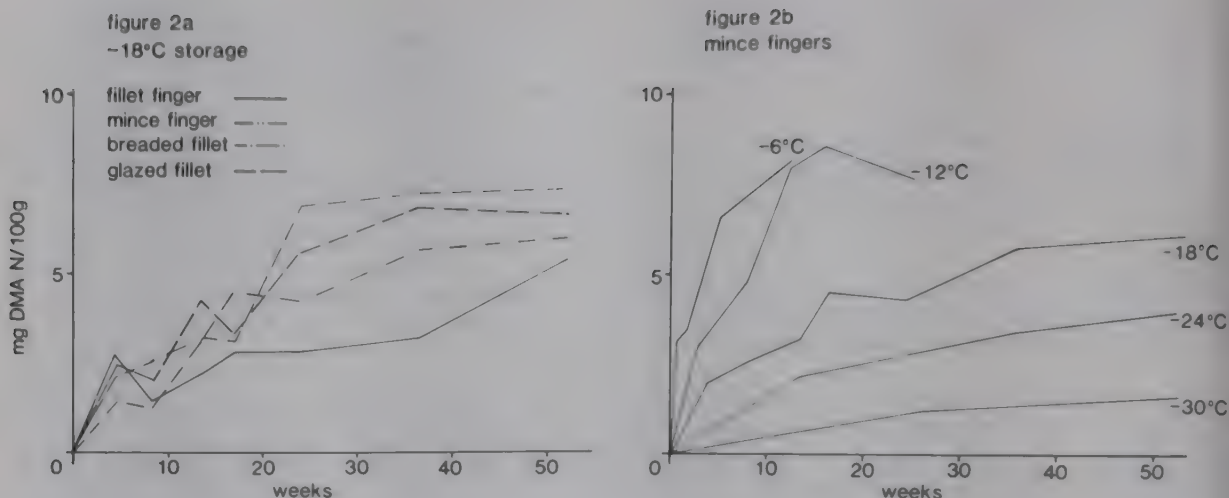


Fig. 2 - Dimethylamine content of frozen cod products

The main conclusion to be drawn is that, although extensive organoleptic and chemical changes may take place during frozen storage, the acceptability of consumer products of the types tested remains essentially unchanged even after 1 year at -18°C .

REFERENCES

1. COUNCIL OF THE EUROPEAN COMMUNITIES: Council Directive of 18 December 1978 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs for sale to the ultimate consumer (79/112/EEC). Official Journal of the European Communities No. L 33 (1979) 1-15.
2. P. HOWGATE : Approaches to the definition and measurement of the storage life of chilled and frozen fish. Proceedings of this Conference.
3. T.P. LABUZA : Shelf life dating of foods. Food and Nutrition Press (1982).
4. D.R. PERYAM and F.J. PILGRIM : Hedonic scale method of measuring food preferences. Food Technol. 11 (1957) pp. 9-14 (Supplement).
5. W.M. LAIRD, I.M. MACKIE and J. REGENSTEIN : Deterioration of frozen cod and haddock minces. I.I.R. Commission C2. Boston, USA (1981).
6. P. HOWGATE : Sensory properties of minced cod and herring. In 'The Production and Utilization of Mechanically Recovered Fish Flesh' J.N. Keay ed., Torry Research Station, Aberdeen 1976, pp. 49-53.
7. J.J. CONNELL and P. HOWGATE : Consumer evaluation of fresh and frozen fish. In "Fish Inspection and Quality Control", R. Kreuzer, ed. Fishing News (Books) Ltd, Surrey, England (1971), pp. 155-159.

ACCEPTABILITE DU POISSON CONGELE VENDU AU DETAIL

RESUME : On présente les résultats de la mesure de l'acceptabilité par le consommateur de quatre produits à base de poisson vendus au détail, au cours d'un entreposage atteignant un an à -18°C . Les produits, choisis d'après leur valeur marchande au Royaume-Uni, étaient des filets de cabillaud glacés, des filets de cabillaud panés, des bâtonnets de filet de cabillaud et des bâtonnets de hachis de cabillaud. Pour réaliser sur ces produits une consultation plus large qu'il n'était possible à la Torry Research Station, un projet a été établi en collaboration avec neuf collègues d'économie domestique dans tout le Royaume-Uni avec 350 à 400 assesseurs. Des échantillons ont été prélevés périodiquement et évalués selon la méthode de l'échelle hédonique par des jurys de dégustation des collègues, des jurys de dégustation et des jurys entraînés de la Torry Research Station, ainsi que par détermination de la teneur en diméthylamine. Les résultats montrent que, malgré une nette augmentation de la dureté de la saveur d'entreposage et de la teneur en diméthylamine, l'acceptabilité des produits dans les conditions particulières de l'évaluation ne présentait pas de différences notables au cours de l'année.

On examine les implications de ces résultats en se référant au problème de l'établissement de durées de conservation raisonnables pour les produits à base de poisson entreposés dans des conditions commerciales.

DISCUSSION

A. BREMNER (Australia) - Have you an explanation for the low scores given to all the samples by the Torry Hedonic Panel (Fig 1c) and to the fact that fish fingers made from mince were given the same ratings as the fillets ?

I.M. MACKIE - I have no obvious explanation for the low scores given by the Torry hedonic panel for all the samples at the start of the storage trial. We made a particular point of selecting fish of high initial quality ie: a freshness score 8.0 or higher on the Torry descriptive scoring system and we decided to start the project in October when the fish would have recovered from spawning in early summer. Nonetheless, the fish were rated softer than normal by the trained taste panel and it may be that this has been an important factor in the hedonic assessments. The increase in liking in the early stages of storage could possibly be due to a firming of the flesh as a result of frozen storage. It may be that this downgrading of the fish initially was more pronounced in the fillets and fillet finger than in the mince finger which, in any case, has a different texture. At the later stage of storage the mince fingers were consistently rated less acceptable than the other products by the Torry and the college panels.

H. HOUWING (Netherlands) - I do not understand why glazed fillets are less appreciated than, for instance, fillet fingers or breaded fillets. Not only was the overall appreciation lower but toughness and cold storage flavour markings were higher. The same applies with respect to DMA formation.

I.M. MACKIE - It is certainly true that the overall college ratings for glazed fillet are lower than for the other two products but, since the TRS hedonic scores do not differentiate between them, too much should not be read into these apparent differences. Taking account of the normal 'noise' or 'scatter', TRS trained panel scores for toughness and cold store flavour do not differentiate between the products. I would make the same comment about DMA values; there is no significant difference if the known error in the method is taken into account. Any difference is unlikely to be due to differences in the raw material used since the same fish were used to prepare different products. It is possible that breeding gives a better protection than glazing but this is not supported by the scores from the TRS hedonic panel.

MILK POWDERS AS CRYOPROTECTANTS IN FROZEN MINCED FISH

Jette Nielsen

Alice Bruun

Technological Laboratory, Danish Ministry of Fisheries

Technical University

Lyngby (Denmark)

Annette Madsen

Atlas-Denmark A/S

Ballerup (Denmark)

1. INTRODUCTION

In order to prevent freeze denaturation, anti-denaturing agents such as polyphosphates and sugars in the form of sucrose and lactose are often added to minced fish. Different salts, protein hydrolysates and various amino acids are also reported as having a cryoprotective effect /1/.

In traditional Scandinavian recipes, milk powder is used in minced fish dishes and it has been known that the functional properties of frozen minced fish are protected during freezing when a rather high amount (10-12%) is added before freezing /2/. At our laboratory it was shown that 8% skimmilk powder can replace a mixture of 4% sorbitol and 4% sucrose in Surimi (Japanese fish paste) without giving the sweet taste usually connected with Surimi /3/. The comparison was made as a double folding test on a Kamaboko product.

In order to improve the texture and to whiten the colour of minced blue whiting, 10% skimmilk powder was mixed into the fish. The mince was then drum-frozen, granulated and extruded into fish sticks. After frozen storage, the fish sticks had a good texture and were whiter than a reference, but they had a slight sweet off-flavour /4/.

The present work compares the cryoprotective effect of skimmilk powder and other milk protein powders in different concentrations with that obtained from lactose and fish protein hydrolysates.

2. MATERIALS AND METHODS

Fillets of cod (Gadus morhua) from the Baltic Sea were minced. The minced fish was frozen in either a plate freezer or in a freezing tunnel (-40°C) and cut into portions which were kept in polyethylene bags at -10 or -12°C for accelerated frozen storage studies. The minced cod was mixed with a suspension containing lactose and milk protein powders.

The minced cod was also mixed with two fish hydrolysates. The hydrolysate was cod fillet waste hydrolysed with NOVO Alcalase 0.6 L /5/. Hydrolysate 331 was spray-dried to a dry matter content of 96.7% and a salt content of 2.4%. Hydrolysate 414 was freeze-dried to a dry matter content of 95.2% and a salt content of 1.0%. The degree of hydrolysis was 8-10% DH.

Total volatile TVN was determined according to /6/, water holding capacity according to /7/. Thaw drip was measured in small blocks of 10 x 5 x 2 cm. The blocks were placed in a net over a tray and the blocks were thawed at 10°C overnight protected from dehydration by polyethylene.

To test the textural characteristics, especially the gelling abilities of the mince, a fish ball model product was prepared in a Robot Coupé mixer. The model product was always prepared with the same final content of dry matter, as the compressive strength necessary for breaking the product is highly dependent on the dry matter content.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

	Protein %	Lactose %	Fat %	Ash %
Skimmilk powder (SFK, Denmark)	36.5	50.5	1.0	8.0
Whey protein concentrate				
Lacprodan 60 (Denmark Protein A/S)	60.0	23.0	6.0	4.0
Lactose (Bie & Berntsen, Denmark)		100		
Whey powder (FSK, Denmark)	17.5	71.0	1.5	8.0
EM-HV Caseinat*	94.3	0.2	0.9	4.7
EM 6 Caseinat*	94.6	0.2	0.9	4.5
REFIT-HDA milkprotein*	93.5	0.5	1.6	4.1

* From De Melkeindustrie Veghel BV, Holland

TABLE 1 - List of ingredients

The formula, a modified original Scandinavian recipe, is based on 300 g fish. A g fish, 12.75 g salt, B g ice or ice water, 33.69 g skimmilk powder, 19.05 g potato starch, 19.05 g wheat flour, 0.36 g pepper. (B is 561-A, A = 5850/% dry matter in the fish). After mixing, the batter was stuffed into a casing and evacuated before closing. The sausage diameter is 4.5 cm and must be no longer than 15 cm. The sausage was heat-processed for 30 min. at 90°C and stored overnight at 0-2°C before testing.

The testing was performed in an Instron 1140 testing machine using a cylindrical piston. A cylindrical sample, 25 mm in diameter and 15 mm high, was cut from the sausage. The force/deformation curve was recorded and the compressive strength of the gel was determined as the maximum force generated before breaking. The compressive strength can be measured in N or in N/cm². Fish of poor quality give a soft gel, which results in a low compressive strength.

3. RESULTS AND DISCUSSION

Experiment 1: Minced cod fillets were gently mixed with a suspension of milk protein and lactose. The various ingredients were added as substitutes for fish dry matter in two different concentrations. Different concentrations of the powders were added in suspensions with a dry matter content of 18%.

TVN of the fresh cod was 10.9 mg N/100 g. The dry matter content was 17.9%.

Powder added to the minced fish	g powder/g fish	Dry matter in mixture %	Compressive strength N	
			Before freezing	After 1 month at -10°C
Lactose	0.02	17.5	-	10.5
	0.11	16.8	17.9	3.3
Skimmilkpowder	0.02	17.6	-	11.6
	0.11	16.8	21.2	5.8
REFIT-HDA	0.02	17.8	34.5	4.1
	0.11	17.6	22.0	1.5
EM 6	0.02	17.4	29.5	4.2
	0.11	18.3	13.2	-
Whey powder	0.02	17.8	36.6	7.3
	0.11	17.3	17.8	3.5
Fish	-	17.9	47.0	1.1

TABLE 2 - Compressive strength

During storage for 3 weeks at -30°C there was no development in TVN.

Powder added to the minced fish	Thaw drip %	WHC %	TVN mg N/100 g
Lactose	14.2 26.1	49.0 32.0	14.4 10.0
Skimmilk powder	16.6 15.7	48.5 47.6	19.6 11.2
REFIT HDA	15.2 10.2	45.7 35.8	17.2 15.7
EM 6	9.0 14.2	48.6 47.1	15.7 15.9
Whey powder	7.9 28.0	45.1 30.5	19.8 14.9
Fish	10.9	43.4	22.7

TABLE 3 - Analyses after 1 month storage at -10°C

It was seen that the high concentration level of added dry matter had a negative effect on the compressive strength before and after frozen storage. Fresh fish without any additions had the best gelling properties; but after freezing it seemed as if all the added ingredients had a cryoprotective effect regarding gelling properties. Lactose and skimmilk powder showed the best effect. There was no marked influence of the additives on TVN regarding diluting effect. High concentrations of lactose and whey powder had a negative effect on thaw drip. Low concentrations of EM6 and whey powder showed no negative influence.

The analysis of WHC showed low values for high concentrations of lactose, REFIT-HDA and whey powder and acceptable values for the rest, with a small positive influence of low concentrations of lactose, EM6 and of low and high concentrations of skimmilk powder.

Experiment 2: Experiment 2 was performed exactly as experiment 1. The fillets were in a rather bad condition with a TVN as high as 23.5 mg N/100 g and a dry matter content of 18%. There was no development in the content of TVN during frozen storage at -10°C for 3 weeks.

Powder added to the minced fish	g powder/g fish	Dry matter in mixture %	Compressive strength N	
			Before freezing	After 3 weeks at -10°C
Lacprodan	0.02	17.6	52.4	14.3
	0.05	16.9	38.3	14.8
Whey powder	0.01	17.8	27.5	11.8
	0.04	16.3	19.6	13.3
Skimmilk powder	0.02	17.4	33.1	13.0
	0.05	16.5	27.8	15.3
DM-HV	0.02	17.6	34.7	12.2
	0.04	16.6	22.0	11.4
Hydrolysate 331	0.02	17.5	30.5	9.2
	0.04	17.2	25.8	8.3
Hydrolysate 414	0.02	17.5	30.3	7.1
	0.04	17.4	22.3	11.9

TABLE 4 - Compressive strength

The concentration levels of the additives had almost no influence on the compressive strength. The milk powder showed an overall better effect on the gelling strength than fish hydrolysate. Lacprodan-60 in both concentrations and skimmilk powder gave the best result. The WHC for the high concentration of 331 was very good and the compressive strength was acceptable. Lacprodan-60 and whey powder in the high concentration gave a low WHC both before and after freezing. All the samples of experiment 2 were also stored at -25°C and will be analysed after 3 months of frozen storage.

Experiments 1 and 2 showed an antidenaturing effect of 2% lactose but also of whey powder, skimmilk powder, whey concentrate and even of caseinate EM-HV. The lactose content in the additives varied from 0.2-71.0% (see table 1). This indicates that it is not only the lactose which is the cause of the antidenaturing effect.

Milk proteins are reported as having antioxidative effects /8/ and part of the effect might be due to this. However, in these experiments there has been no significant difference in the TBA values measured after frozen storage.

Another hypotheses often suggested as the cause of denaturation is the reaction of formaldehyde developed under frozen storage with fish protein. To prevent this reaction, milk protein is added, as this protein may react with formaldehyde instead of formaldehyde reacting with the fish protein. In experiment 2, however, there was no development in the TVN value. This indicated that no dimethylamine and thereby no formaldehyde were formed, although a cryoprotective effect of the additives was seen.

Matsumoto /1/ suggests that a cryoprotectant-protein complex is formed by ion- or hydrogen bonds. Water remains linked in the complex during freezing, thus limiting the formation of ice crystals, but also together with the cryoprotectants, preventing the fish protein from aggregation and thereby denaturation. Matsumoto's theory, however, comprises only small molecules like amino acids, salts and polyphosphates.

None of the hypotheses mentioned can fully explain the effect of the milk proteins, and further experimental work is needed.

Experiment 3: The antidenaturing effect of different concentrations of skimmilk powder was further investigated. Skimmilk powder was mixed into the minced fish in three different ways. A) as a substitute for a certain amount of fish dry matter as in experiments 1 and 2; B) as dry powder without further addition of water; C) as a powder in different concentrations suspended in the same amount of water. 15% water was added.

When making the model product in A the skimmilk powder indicated in the formula was added as if the different mixtures consisted of fish only. In B and C an amount of skimmilk powder corresponding to the difference between that given in the formula and that mixed into the fish raw material was added. In all cases the final dry matter content of the model product was the same.

The preliminary results in experiment A (table 5) show an optimum effect by the addition of 3 to 4% of skimmilk powder after 3 weeks of frozen storage. This corresponds to the addition of approx. 17.4-21.9 g skimmilk powder per g of total dry matter. The raw material in experiment A had a high TVN. In experiments B and C (table 6) the raw material was of a good quality, but the time of storage was 2 months. This gave a rather low value of compressive strength. The result indicates, however, that the optimal concentration of skimmilk powder is 6-8% corresponding to approx. 30-40 g added skimmilk powder per 100 g of total dry matter.

A: Minced cod fillets from experiment 2 were used.

g skim milk powder/g fish	g skim milk powder/100 g total dry matter	Total dry matter %	After storage for 3 weeks at -10°C Thaw drip %	Compressive strength N
0.01	5.05	18.2	1.4	15.3
0.02	11.3	17.7	2.8	13.5
0.03	17.4	17.2	8.3	18.3
0.04	21.9	18.2	2.0	16.6
0.06	37.5	16.0	18.6	12.9
Fish		16.8	11.1	12.7

TABLE 5 - Addition of different concentrations of skim milk powder in suspensions with a dry matter content of 18%.

B and C: Minced cod fillets with a TVN of 11.7 mg N/100 g were used. The storage was at -12°C for 2 months.

g skim milk powder/g fish	g skim milk powder/100 g total dry matter	Compressive strength N	g skim milk powder/100 g total dry matter	Compressive strength N
0.02	9.9		12.0	1.02
0.04	19.3	0.82	22.6	1.36
0.06	29.4	2.62	31.8	2.92
0.07	32.4	5.00	36.5	3.62
0.08	35.2	4.52	40.6	3.70
0.09	39.0	3.60	44.6	3.00
0.10	41.3	3.60	48.5	3.20
0.12	48.0	4.08	55.8	2.96
Fish	-	0.82		-
Fish added 15% water				0.66

TABLE 6 - Addition of different concentrations of skim milk powder to fish (B) and fish with an addition of 15% water (C).

The experiments with different concentrations of skim milk indicate that the optimum concentration of skim milk powder might be dependent of the raw material, the time of storage and the mixing method.

REFERENCES:

- /1/ MATSUMOTO, J.J.: Chemical deterioration of muscle protein during frozen storage. In: Chemical Deteriorations of Protein. J.R. Whitaker & M. Fujimati (eds.). Symposium Series 123. American Chemical Society, 1980.
- /2/ BRUUN, A., D.V. DALSGÅRD, P. IKKALA, & A. VILLADSEN: Undersøgelse af separeret fiskekøds holdbarhed som råvare til fremstilling af fiskefars. Report of the Technological Laboratory of the Danish Ministry of Fisheries, Lyngby, 1976.
- /3/ NIELSEN, J. & A. MADSEN: Unpublished results, 1984.

- /4/ MADSEN, A.: Forbedring af processen til fremstilling af frossen fish ud fra mindre fiskearter og biprodukter fra filetproduktionen. Thesis submitted to the Academy of Technical Sciences, Denmark, 1984.
- /5/ NIELSEN, J.: Enzyme hydrolysates as stabilizers in frozen fish? Paper presented at the WEFTA meeting in Ijmuiden, Holland, 1983.
- /6/ CONWAY, E.J. & A. BYRNE: An absorption apparatus for the micro-determination of ammonia. *Journal of Biochemistry* 27, 419-429, 1933.
- /7/ BØRRESEN, T.: Nyutviklede metoder for bedømmelse av vandbindings-evne, saltvandsbindingsevne og koketab i fiskemel. Rapport nr. 663.1-7-2. Fiskeriteknologisk Forsknings-institutt, Tromsø, Norge, 1980.
- /8/ TAYLOR, M.J., T. RICHARDSON: Antioxidant activity of skimmilk: effect of heat and resultant sulfhydryl groups. *Journal of Dairy Science* 63(11) 1783-1795 (1980)

LES POUDRES DE LAIT COMME CRYOPROTECTEURS DES HACHIS DE POISSON CONGELE

RESUME : Le développement de l'utilisation du hachis de poisson en remplacement des filets, en blocs de filets congelés et en blocs de hachis, est limité en raison de leur texture sèche et coriace, de leur goût plat ou encore de leur couleur non blanche.

Souvent, en vue d'empêcher la dénaturation du hachis du poisson, des polyphosphates et divers alcools de sucre sont ajoutés avant congélation. On a également signalé d'autres additifs ayant un effet cryoprotecteur comme par exemple des amino-acides. Dans les recettes scandinaves traditionnelles, la poudre de lait est employée dans des préparations de hachis de poisson et il est établi, depuis plusieurs années, que les propriétés culinaires des hachis de poisson congelé sont protégées quand il en est ajouté une quantité plutôt importante (8 à 10 %) avant congélation.

Le travail présenté concerne l'activité cryoprotectrice des poudres de protéines de lait, à différentes concentrations, en comparaison avec ce qui est obtenu par addition de lactose et d'hydrolysats d'enzymes. La température de stockage a été - 10°C et - 25°C. L'étude de l'effet cryoprotecteur a été fait par la mesure Instron de la texture, par des tests sensoriels et par des analyses chimiques. Il est apparu que des poudres de protéines de lait agissent sur le goût, la couleur et les propriétés culinaires. On estime que ceci peut être dû, en partie, à la présence de lactose.

DISCUSSION

P. HOWGATE (U.K.) - It is not surprising that there is no texture change after 3 months storage at -25°C. This is a mild treatment and both sensory and instrumental methods have shown that there is no appreciable change during short period storage at a temperature of -25°C.

J. NIELSEN - Our Instron method measures gelling ability, not texture. Normally the gelling ability of minced fish decreases rapidly during frozen storage; even below -25°C.

SECTION V

PROCÉDÉS TENDANT À PROLONGER
L'ENTREPOSAGE DES PRODUITS RÉFRIGÉRÉS

*POSSIBLE METHODS OF EXTENDING CHILLED
STORAGE LIFE*

RADIATION PRESERVATION OF FISH

G. HOBBS
Torry Research Station, Aberdeen
F.J. LEY
Isotron Ltd, Swindon (United Kingdom)

FISH LANDINGS IN THE UK

Approximately 839 k tonnes (1982 figures) of fish and shellfish are landed in the UK each year. This is comprised of 501 k tonnes of demersal fish which are largely non-fatty, 273 k tonnes of pelagic fish which mostly have a high fat content, and 65 k tonnes of shellfish. The first sale values of these three groups of fish are £218 M, £29 M and £43 M respectively. The value added to this fish by the time it reaches the consumer is very varied depending on the type of product sold; however the total is of the order of £1200 M.

In addition, imports of chilled or frozen demersal fish add a further 115 k tonnes (value £68 M), pelagic fish a further 10 k tonnes (value £4 M) and shellfish a further 16 k tonnes (value £51 M). These figures do not include salmon and trout, nor fully preserved imports such as canned fish. In addition, 89 k tonnes of frozen fillets (value £109 M) are imported.

Not all of this fish is distributed in such a way that it would benefit from a process such as irradiation. Fatty fish will be adversely affected by accelerated oxidation processes, though this may be acceptable in the case of smoked products for instance. Fish distributed unpackaged to the consumer for instance through the traditional fishmonger's shop would be a doubtful application since there is the possibility of recontamination with bacteria after processing and any advantages may be lost. There is, however, a considerable amount of fish and shellfish distributed packaged, both frozen and chill stored, which could benefit from irradiation, and indeed the advantages achieved could provide opportunities to substantially increase the amount of fish distributed in this way.

Fish would preferably be irradiated in the final catering or consumer pack. Thus if, for example, all the demersal fish landed in the UK were available, this would yield about 200 k tonnes of fillets. As will be seen later, the advantages of irradiation are only fully realised if good quality fish is treated. Whilst no precise figure can at present be put on this, it is likely that more than 70% of the UK landings of demersal fish and most of the shellfish would be suitable.

Irradiation essentially kills bacteria and there are two purposes to which the process can be put. Firstly, it can be used to extend the shelf life of chilled stored fish. Secondly, it can be used to eliminate pathogenic bacteria from chilled or frozen fish. The process can thus have an economic advantage by easing distribution costs, improve the quality of fish available to the consumer and reduce public health risks.

SPOILAGE OF FISH

The eating quality of chill stored fish and shellfish depends primarily on its freshness, though acceptability is subjective and will vary. Immediately post mortem the changes that occur in chill stored fish are a combination of chemical and enzymic changes, generally termed autolysis. These result chiefly in the loss of freshness characteristics and are followed by changes resulting from the growth of bacteria which eventually lead to putrefaction. With few exceptions (fat oxidation being the main one), the undesirable changes occurring are due to bacterial growth and metabolism. Detailed discussions of microbial spoilage of fish have recently been published /1/ /2/.

The bacteria which cause spoilage of fish at refrigeration temperatures are Gram negative psychrophilic or psychrotrophic organisms of the genera *Pseudomonas*, *Alteromonas*, *Moraxella* and *Acinetobacter*, strains belonging to the first two genera being the most active spoilers. Whilst refrigeration slows down the growth of spoilage bacteria, they are capable of growth at 0°C or somewhat below.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

When fish are freshly caught bacteria are present on the skin and in the intestines. The flora is quite varied, however, the same few genera tend to predominate. Typical examples are given in Table I. Whatever the initial flora, during storage at or near ice temperature, the Gram negative psychrophilic organisms eventually predominate. A typical spoilage pattern during ice storage is shown in Table II.

TABLE I
Bacterial flora of fresh fish
(percentages of total flora)

	Cod	Herring	Skate	Lemon sole	Scampi
<i>Pseudomonas</i>) <i>Alteromonas</i>)	47.5	32.2	52.5	60.3	18.0
<i>Moraxella</i>) <i>Acinetobacter</i>)	36.6	26.6	18.8	22.3	39.0
<i>Vibrio</i>	4.7	2.6	12.5	8.7	-
<i>Flavobacterium</i>) <i>Cytophaga</i>)	3.9	17.6	9.4	5.0	6.0
<i>Coryneform</i>) <i>Micrococcus</i>)	6.0	11.5	4.9	2.3	34.0

TABLE II
Changes in the flora of ice-stored cod
(percentages of total flora)

	0 days	5 days	10 days	15 days
<i>Pseudomonas</i>) <i>Alteromonas</i>)	14	17	50	82
<i>Moraxella</i>) <i>Acinetobacter</i>)	33	49	38	14
<i>Flavobacterium</i>) <i>Cytophaga</i>)	4	0	0	2
<i>Coryneform</i>) <i>Micrococcus</i>)	49	34	12	2

FOOD POISONING FROM FISH

Fish and shellfish are, in the UK at least, amongst the safest articles of diet /3/. Nevertheless they can, especially when handled in an unhygienic manner, cause most of the well established types of food poisoning. In addition, toxic species of fish occur and toxins are accumulated in live fish from their feed. These will not be considered further since they are not generally affected by irradiation, nor do they increase during storage.

Fish caught in the open sea are generally free of food poisoning bacteria. There are, however, three situations where bacteria which are normally present in the aquatic environment have caused problems. *Clostridium botulinum* and *Vibrio parahaemolyticus*

are sometimes present in fish when caught and scombrototoxin poisoning is generally thought to result from bacterial growth though the precise nature of the toxin is not known nor has it been associated with specific microorganisms.

Historically, sewage pollution has raised problems with coastal fisheries for shellfish. This has been controlled, in the case of molluscan shellfish, by the use of well established depuration processes. In recent years, however, it has become apparent that in some parts of the world commercial fisheries are close enough inshore to be affected by contamination from sewage outfalls. In such areas *Salmonella* spp. can be expected from freshly caught fish. In addition, there is an increasing awareness that certain viruses can be transmitted, particularly by shellfish. Again, the contamination arises from sewage pollution.

With few exceptions contamination of the fish alone is not sufficient to result in food poisoning, in general the numbers of contaminating bacteria are too small. In the case of *Salmonella*, *Vibrio* and *Clostridium perfringens* poisoning, the organisms grow and produce an infection in the patient's intestines. However, some growth in the fish before consumption is usually necessary to produce an infective dose. Under conditions of poor hygiene, it is possible, though rare, for contamination with sufficient numbers to occur. In the case of *Clostridium botulinum* and *Staphylococcus aureus* poisoning results from the ingestion of toxins. Growth of the organisms in the fish before consumption is therefore a prerequisite.

As has already been pointed out, the conditions leading to poisoning usually result from mishandling. With the exception of some strains of *Clostridium botulinum* which can grow at about 4°C, the minimum growth temperature for food poisoning bacteria is 8°C or higher.

IRRADIATION PROCESSING

It has been established for some time that sterilization of fishery products by irradiation using the equivalent of a 12D heat process, 40-60 kGy (4-6 Mrad), results in unacceptable organoleptic changes in fish. These flavours and odours are produced by the direct and indirect action of irradiation on chemical components. Some partial success in controlling these changes has been achieved by processing at low temperatures, especially below -20°C. Also, synergism between irradiation and other agents such as heat and chemical preservatives has been demonstrated, thus permitting the use of lower doses. At present none of these processes are entirely satisfactory and hence are not commercially viable. With most fishery products the maximum dose which does not produce undesirable changes is about 3 kGy (0.3 Mrad). Such a radiation pasteurization or radurization process does not sterilize fish but it can provide an extension of shelf life of 2 or 3 times. This dose is well within the 10 kGy (1.0 Mrad) average dose limit proposed with respect to the safety of irradiated foods /4/. As with other methods of pasteurization, the main benefits of low dose irradiation are achieved when the process is combined with efficient refrigerated storage and the selection for processing of good quality fish. Unlike heat processing, however, it has little effect on the enzyme systems of the fish and hence does not slow down the autolytic changes.

The effects of radiation processing on the microbiological status of a product will depend on the relative sensitivities of the various organisms present /5/. Examples of the sensitivity of bacteria to irradiation are given in Fig. 1.

CONTROL OF SPOILAGE

A dose of 3 kGy (0.3 Mrad) reduces the total number of bacteria on fish by a factor of about 10^3 . Fortunately strains of *Pseudomonas* and *Alteromonas* are significantly more sensitive to irradiation than those of other genera present, hence proportionally more of the active 'spoilage' bacteria are killed.

The predominant organisms surviving such a process belong to the genera *Moraxella*, *Acinetobacter* and *Micrococcus* and to the coryneform group. The last two groups do not grow well at 0°C, hence the flora developing on chill stored, radiation pasteurized fish is composed chiefly of *Moraxella* and *Acinetobacter* strains. Whilst these bacteria grow in chill stored fish they produce less offensive organoleptic changes than the normal spoilage bacteria. *Pseudomonas* and *Alteromonas* can become significant in the later stages of spoilage depending on the number surviving processing.

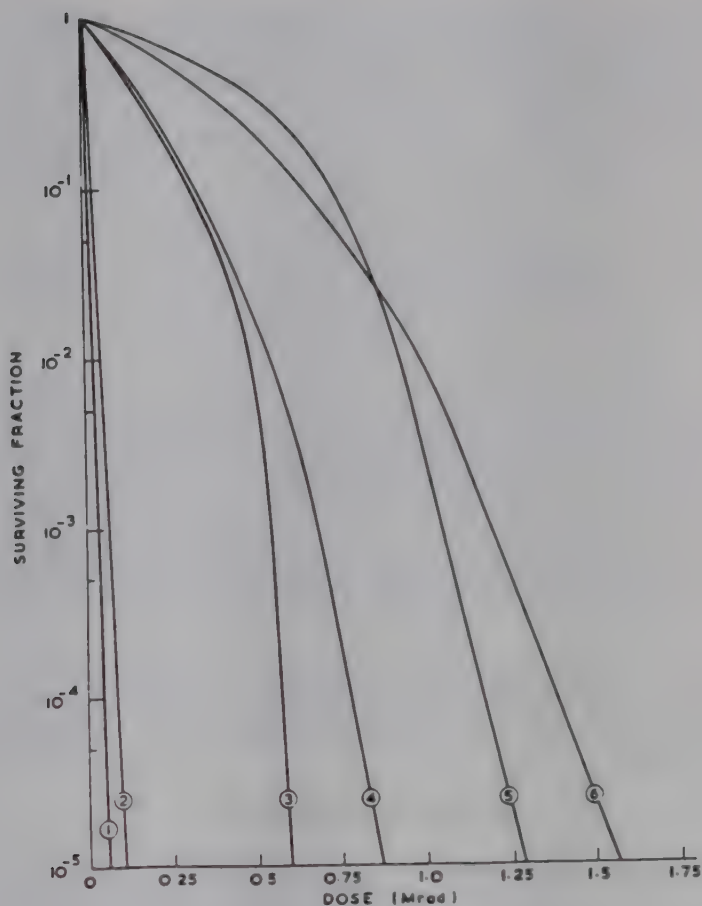


Fig. 1 - Typical dose/survival curves for some bacteria irradiated in buffered suspensions in the presence of air

1. *Pseudomonas*, *Alteromonas* and *Vibrio* spp., *E. coli*
2. *Salmonella*, *Acinetobacter* and *Moraxella* spp.
3. *Streptococcus faecalis*
4. Aerobic spores
5. *Clostridium botulinum* spores
6. *Micrococcus radiodurans*

Thus the shelf life extension resulting from the radiation pasteurization of fish is a result of the reduction in total number of bacteria but, primarily, the preferential reduction of active 'spoilage'. For reasons which are self evident, the processing and subsequent storage of unpackaged fish is somewhat less effective since recontamination with spoilage bacteria can occur.

Examples of the shelf life extensions that have been reported for fishery products are given in Table III and an example of the extension of good quality shelf life in Table IV.

The results in Table IV also demonstrate the loss of advantage of irradiation if poor quality fish are processed. The full benefits of the process can be realized only with fresh fish and the practice of good chilling and hygiene.

Further benefits can be achieved if the irradiation process is combined with some other procedure such as heating. For example, Hannesson and Dagbjartsson /6/ showed that the shelf life of lobster tails was more than doubled using a combination of irradiation and blanching as opposed to irradiation alone. The additional effects result from synergism between heating and irradiation and the inactivation of autolytic enzymes by blanching.

TABLE III

Shelf life extensions achieved with cod fillets by irradiation
(factor x unirradiated shelf life)

Laboratory	Dose (kGy)	Storage Temperature				
		10°C	7.0-7.8°C	5.6°C	3.3°C	0.3°C
1	1	1.4	2.0	2.6		3.0
	2	2.0	3.0	3.6		4.0
2(a)	1	2.2	2.4	2.9		
	2	3.0	3.0	3.4		
2(b)	1	1.8	1.8			
	2	1.9	1.9			
3(a)	1	2.4	2.4		2.2	
	2	2.4	4.4		3.0	
3(b)	1	1.8	1.8			
	2	2.3	2.6			
4	1	1.8				
	2	2.8				
5	3	2.0-3.0			2.3	

TABLE IV

Effect of irradiation (3 kGy) on the shelf life
of cod stored at 3.3°C

Days on ice before packing	Shelf life to score of 4.5* (days)		Shelf life to score of 6.0* (days)	
	Not Irradiated	Irradiated	Not Irradiated	Irradiated
2	13	30	8	22
4	11	26	6	17
6	9	22	4	11
8	7	18	2	6
10	5	14	0	0
12	3	8	0	0
14	0	0	0	0

*Raw odour score in taste panel assessment

ELIMINATION OF PATHOGENS

The Gram negative bacteria belonging to the *Salmonella* and *Vibrio* genera are relatively sensitive to irradiation (cf. Fig. 1). Provided they are not present in large numbers, it is clear that relatively small doses of irradiation will suffice to eliminate them from fishery products. Whilst it would be preferable to exclude such food poisoning bacteria from the raw material, there are clearly some situations where this is not always possible. Such an example is the occurrence of both *Salmonella* spp. and *Vibrio parahaemolyticus* in Malaysian frozen cooked prawns

imported into the UK /7/. These prawns are imported in bulk through one or two ports and irradiation of a product such as this would be a simple matter logistically. They could be irradiated in the frozen state, eliminating the pathogenic bacteria with no detectable effects on the quality of the product. In Australia batches of frozen shrimps amounting to some 60 tonnes were successfully irradiated in the frozen state using doses of 6-8 kGy (0.6-0.8 Mrad) /8/. It should be noted that micro-organisms are more resistant in frozen foods than at chill or ambient temperature.

Clostridium botulinum presents a rather different problem. Compared to the Gram negative bacteria, the spores of this organism are resistant to irradiation. A dose of 3 kGy (0.3 Mrad) would reduce the spore load on the fish by a factor of 10 or less. Because the incidence and numbers of these spores on fish are usually low, the process will eliminate them in many cases. It could not, however, be relied upon to do so and the rate of subsequent outgrowth and toxin production will be little different from that in the untreated product where survivors do occur.

Examples of the rate of toxin production and its relation to the shelf life of the product are shown in Table V. Where conditions of abuse exist, particularly

TABLE V
Relationship between shelf life and toxin production by
Cl. botulinum type E (10^6 spores g^{-1}) [days]

Species	Dose (kGy)	20°C		10°C		5°C	
		Shelf life*	Toxin	Shelf life*	Toxin	Shelf life*	Toxin
Cod	0	1		3	5-9	7	
	3	2		6-9	5-9	12-15	
Haddock	0	1		3	5-12	7	
	3	2		6-9	5-12	12-15	
Herring	0	< 1	1-5	1-2	4-7	3-4	12-32
	3	1	1-5	2-4	4-7	6-8	12-32
Kipper	0	5-6	2-8	6-9	8-20	7-10	< 28
	3	10-15	2-8	10-18	8-20	15-20	< 28
Smoked Haddock	0	2-5	3	5-8	28-30	10-12	< 28
	3	4-8	3-4	10-15	28-30	20-25	< 28

*Defined as the time required to reach a Raw Odour score of 4.5 by taste panel assessment

storage at higher temperatures, toxin will accumulate in many products before the end of the shelf life. With good handling and storage, however, botulism will be no more of a hazard than it is with untreated fish. A similar situation exists with fish products (and other foods) which have been heat pasteurized or lightly cooked, especially when they are to be eaten without further cooking as with many smoked products. If combined with good handling and storage, radiation pasteurization will to a limited extent reduce the botulism hazard. Under conditions of gross abuse, however, there could be some increased hazard.

DISTRIBUTION OF IRRADIATED FISH

Introduction of a process such as radiation pasteurization will be an additional cost and must be justified. Where treatment is used to eliminate a possible health risk some increase in production costs can clearly be acceptable. The benefits in using the process to extend shelf life lie in reductions in the distribution costs of chill stored fish and to some extent in the maintenance of good quality for a longer period. Reference to Table IV shows that the refrigerated shelf life of a typical white fish

such as cod is increased about 2½ times by irradiation. Using a taste panel score of 6 (for Raw Odour) and assuming fish will be packed and irradiated no more than 6 days after catching, the minimum shelf life of the pack will be 11 days compared to 4 days without irradiation. To maintain good quality fish in a retail situation requires at present several deliveries each week. With irradiation one delivery each week would be adequate. The logistics of this would require careful consideration since the distribution of chilled fish needs to be integrated into a distribution system for chilled foods generally. Fish is frequently processed and packaged in the same factory as other chilled foods and in that situation radiation processing of other chilled foods could be considered as well. Where fish and shellfish are the only products processed then substantial distribution savings could accrue and, in addition, temporary fluctuations in sales could be accommodated better.

PROCESS CONSIDERATIONS

The radiation under consideration is ionising radiation and limited for practical purposes to gamma rays from the radionuclides cobalt 60 or caesium 137 or electrons by high voltage electrical machines. X-rays from machines are also a possibility for the future.

The development of commercial plants for application involving microbiological control is largely based on the use of gamma radiation from cobalt 60 because it is readily available at low cost and its half-life is reasonably long at 5.3 years. There are currently about 70 megacuries of cobalt 60 employed in some 130 industrial plants throughout the world. Most of this capacity is used for the radiation sterilisation of medical products and about 10 plants are involved in irradiating food.

Cobalt 60 is produced in certain nuclear reactors by bombarding the inactive cobalt 59 with neutrons for periods in excess of one year. The main supplier is Atomic Energy of Canada Limited, but smaller quantities are available in the UK. The size of the sources in commercial plants varies within the range of 100 kilocuries to 5 megacuries, depending on dose requirement and rate of throughput of the product.

The main objective in designing an irradiation plant is to achieve a high absorption of radiation in the product. The dose should be as uniform as possible and the hold-up time in the plant minimal. Figure 2 illustrates a suitable design of 'continuous' plant in which containers of a standard size carry product into a large concrete cell. The safe store position of the source is either a deep-water pond 6 m deep or a shielded trench into which the source is lowered followed by a shielded plug integral with the source frame.

Typical designs use a roller conveyor or monorail system. The containers are pushed by hydraulic or pneumatic rams to and fro across the face of the source and then moved to the other face for similar treatment before emerging. The transfer is so arranged so that half the dose is given through one side of the package and half through the opposite side. The minimum dose is therefore received by the product midway between the two sides. The ratio of maximum to minimum dose, known as the overdose ratio, is determined by the thickness and density of the product.

The costs of the process will depend on the dose required and the rate of throughput. High capital investment in plant, machinery and source indicates continuous usage to achieve an economic process. This implies that a buffer store of product can be maintained. Each potential process requires a feasibility study to establish the type, size and siting of the radiation plant most suitable for the purpose, thus allowing estimation of capital and running costs. However, in broad terms, 1 megacurie of cobalt 60 would process about 10 tonnes per hour at a dose of 2.5 kGy (0.25 Mrad). The source itself would involve an investment of about £800,000 and the plant, shielding and machinery say £1,200,000. A suitable site and additional warehousing would be needed. Calculations of depreciation and operating costs, assuming continuous operation, lead to a process cost in the order of £25.00 per tonne. Small throughput rates require proportionally smaller source strengths - a facility can be built up gradually in say 100,000 curie steps.

LEGAL SITUATION

In the UK, the Food (Control or Irradiation) Regulations 1967, prohibit the use of the process for food for sale for human consumption. However, the government appointed Advisory Committee on Irradiated and Novel Foods is currently reviewing

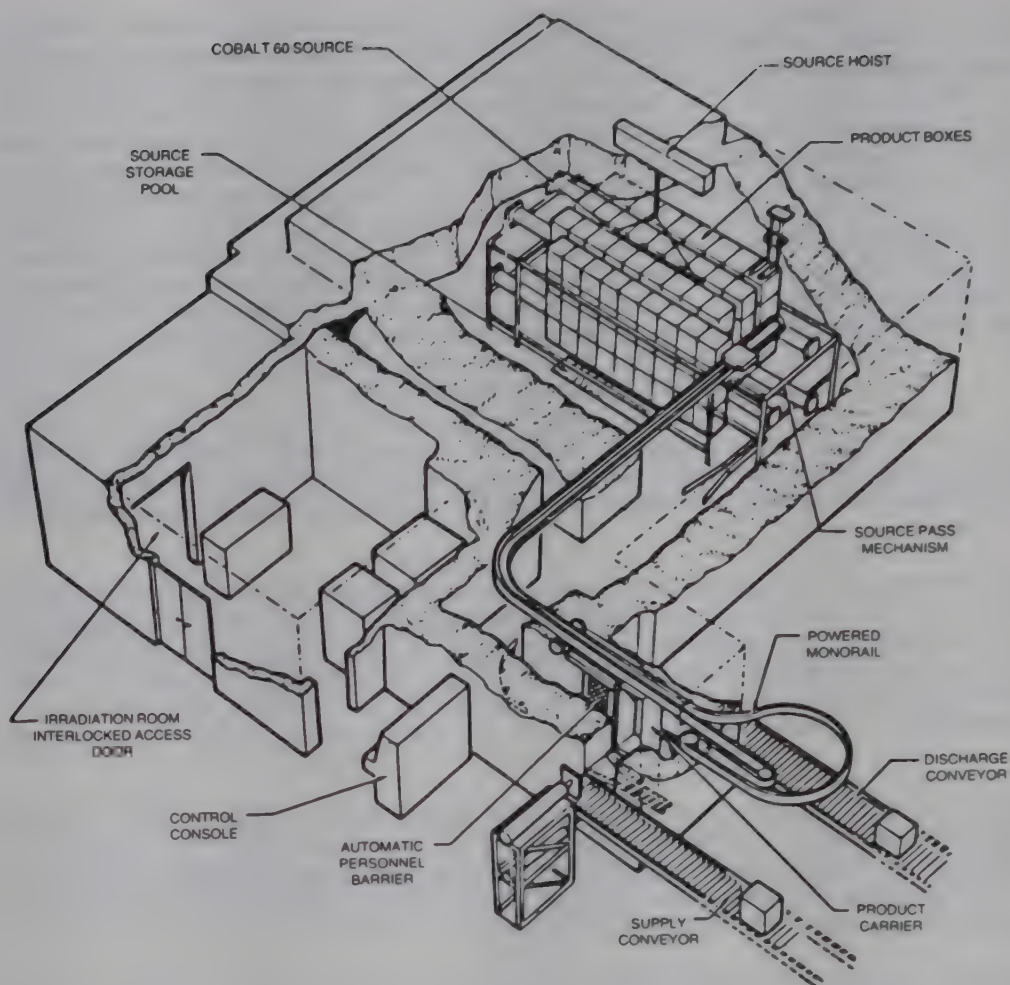


Fig. 2 - Product containers are carried in and out of the concrete cell through a labyrinth. The conveyor is based on either a monorail carrier system as shown or a roller conveyor. The containers follow a route around the source to allow even exposure of product to radiation and its efficient absorption.

evidence on safety and is expected to report this year. If favourable, the regulations could be modified in 1986. Industry is hopeful that a general clearance up to an average dose of 10 kGy (1.0 Mrad) will be given in accord with the UN recommendation in 1981 /4/.

A number of other countries already allow irradiation of food for specific purposes, although application to fish in commercial practice is limited to frozen prawns and shrimps for the purpose of *Salmonella* control. There is no doubt that broader international clearance of irradiated food is needed to stimulate wider use. In the meantime, FAO and WHO have moved ahead to publish through Codex a General Food Standard for Irradiated Foods and a Recommended Code of Practice for the operation of radiation facilities. Consumer reaction in countries already involved has been good, but more effort will be needed to convince industry and the public of the advantages of this new process.

REFERENCES

1. G HOBBS and W HODGKISS (1982) 'The bacteriology of fish handling and processing' In Developments in Food Microbiology - 1 (ed. R. Davies) Applied Science Publishers Ltd, UK, pp 71-117.

2. G HOBBS (1983a) 'Microbial spoilage of fish' In Food Microbiology, Advances and Prospects (eds T A Roberts and F A Skinner) SAB Symposium series No. 11, Academic Press, London, pp 217-219.
3. G HOBBS (1983b) 'Food poisoning and fish' J. Royal Soc. Hlth, 103, 144-149.
4. ANON (1981) 'Wholesomeness of Irradiated Food' Report of a Joint FAO/IAEA/WHO Expert Committee, WHO Tech. Rep. Ser. No. 659 HMSO, London.
5. F J LEY (1973) 'The effect of ionizing radiation on bacteria' In Manual on Radiation Sterilization of Medical and Biological Materials. IAEA Technical Series Report, Serial No. 149, HMSO, London, pp 37-63.
6. G HANNESSON and B DAGBJARTSSON (1970) 'Report on a survey project in Iceland on the use of radiation pasteurization of various seafoods' In Preservation of Fish by Irradiation. Panel Proceedings Series STI/PUB/196, IAEA, Vienna 1970, pp 49-64.
7. P C B TURNBULL and R J GILBERT (1982) 'Fish and Shellfish Poisoning in Britain' In Adverse effects of Foods (eds E F P Jelliffe and D B Jelliffe) Plenum Press, New York, pp 297-306.
8. P A WILLS (1980) 'Commercial application of freezing-irradiation combination processes for pasteurization of two specific batches of cooked, peeled shrimps' Proc. Symp. on Combination Processes in Food Irradiation. Colombo, 24-28 Nov. IAEA STI/PUB/568, Vienna 1981 (Available HMSO, London).

CONSERVATION DU POISSON PAR IRRADIATION.

RESUME : Le traitement des aliments avec des rayons ionisants jusqu'à une dose de 10 kGy ne donne pas de produits toxiques et est généralement considéré comme sûr. Ces doses ne stérilisent pas les aliments mais détruisent un nombre suffisant de bactéries pour prolonger utilement la conservation en magasin de détail. Le poisson peut être irradié à la dose de 3 à 4 kGy sans provoquer de modifications décelables de l'odeur ou de la saveur et, à ce niveau, la conservation en magasin de détail de la plupart des poissons augmente de 2 à 3 fois. De plus, des bactéries pathogènes Gram-négatives telles que les salmonelles et les vibrions sont relativement sensibles à l'irradiation et l'on peut utiliser ce procédé pour les éliminer des produits de la pêche. On examine les paramètres de la préparation et les applications pratiques.

DISCUSSION

H. HOUWING (Netherlands) - Can you explain why a 3 kGy dose is acceptable in the U.K. whereas we found 1 kGy just acceptable and 2 kGy too much? This is important since at 1 kGy the extension of shelf-life is considerably less.

G. HOBBS - Sensory changes resulting from irradiation varies with species and our experience is that with white fish such as cod and haddock there were no detectable changes up to 3 kGy. This would be the maximum dose in the block of irradiated fish with parts of the block being subjected to somewhat less than this. Tolerable doses quoted in the literature are variable ranging between 1 and 10 kGy or even more depending on the product. The maximum would also clearly depend on the sensitivity of different people to irradiation odours and flavours.

H.H. HUSS (Denmark) - With reference to your slide showing the sensitivity of various fish products to the pick up of off odours and flavours due to irradiation, could you say where shrimp would be placed in this respect?

G. HOBBS - There are data in the literature which quote a figure of about 5 kGy as the limit before irradiation odours and flavours can be detected.

L. HERBORG (Denmark) - The use of irradiation to cover up poor hygiene by reducing the count of thermotolerant coliforms is not acceptable. However, could you say what the irradiation dose is for this type of treatment and what the resulting micro-flora will be ?

G. HOBBS - Irradiation is no different in this respect to other processes such as heat pasteurisation, freezing and cold storage and treatment with preservatives and it therefore could be used for this purpose. The resulting microbial flora will depend on that introduced during unhygienic handling. The doses required would be 1-2 kGy and since coliforms are generally sensitive to irradiation they could be easily eliminated with this treatment.

OZONIZED WATER AND ICE TO PRESERVE FRESH FISH

GROUPE (Mycoteroperca bonaci)

R.G. RICE

Rip G. Rice, Incorporated, Ashton, MD (U.S.A.)

W.S. OTWELL

University of Florida, Department of Food Science and Human
Nutrition, Gainesville, FL (U.S.A.)

N. BLAKE

Enviroservices, St. Petersburg, FL (U.S.A.)

D.E. SWEAT

Blue Fin Enterprises, Largo, FL (U.S.A.)

R.L. MARSCHALK

Industrial and Environmental Analysts, Inc.,
Research Triangle Park, NC (U.S.A.)

J.W. FAROUHAR

Food Marketing Institute, Washington, DC (U.S.A.)

1. INTRODUCTION

This study had for its primary objective the evaluation of the concept of washing fresh grouper, a warm water fish, in ozonized water and storing them under ice made from ozonized water. Prior studies of a similar nature conducted on cold water species of fresh salmon /1,2/, squid /3/, jack mackerel and shimaaji /4/, all reported 33% to 50% extensions of shelflife. However, none of these prior studies was conducted with warm water fish; nor did they develop the detailed bacteriological, rancidity, and overall fish quality data required to prove the efficacy of the concept. Finally, no prior study addressed ozone preservation throughout the total fish distribution system, from docking through the retail store stage.

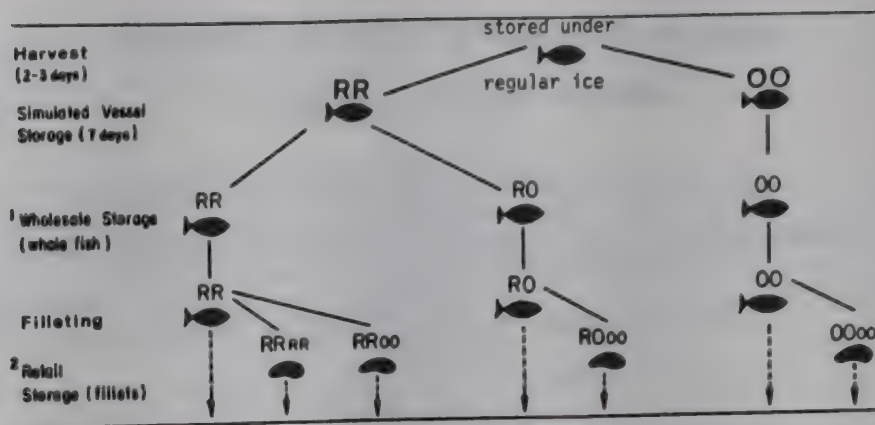
Several earlier studies, reviewed by Otwell et al. /5/, also showed that the onset of rancidity was delayed by application of ozonized water/ice to the fresh fish. On the other hand, Licciardello /6/ reported that the total shelf life of gutted whole cod stored seven days in chilled seawater which was ozonized daily prior to iced storage was no longer than shelf life of cod held in aerated chilled seawater. The use of ozonated ice for on-board storage of Gulf of Mexico shrimp was shown /7/ to provide only a possible one-two days prolongation of life of shrimp.

2. MATERIALS AND METHODS

Black grouper (Mycoteroperca bonaci) was chosen as the primary test fish, based on current availability and similarities in size and composition with other popular, lean varieties of southern species (i.e., Lutianidae-Snappers, Mycoteroperca-Grouper, etc.). All fish came from one harvest utilizing common hook-and-line gear fished in Gulf waters (20 to 40 fathoms) west of Tarpon Springs, Florida. Typically, similar commercial fishing trips land fish two to 14 days after harvest, depending on duration of trip and day of catch. Arrangements were made to assure a short trip, landing grouper after only two to three days from time of catch.

All fish were gutted immediately after catch by customary hand cleaning, which removes viscera and a major portion of the air bladder and blood line (kidney). The head remains intact. In this condition, the fish were stored on regular ice made from the municipal (Tampa) water supply. On return to the dock, the gutted fish were washed (regular or ozonized water) and reiced in either more regular ice or ice made from ozonized water (using the same municipal water supply). This step initiated the basic experimental plan, which is summarized in Figure 1.

Storage and treatment of fish. Fish landed after being stored on regular ice for a maximum of three days remained in the reiced step for an additional seven days, to simulate typical prolonged vessel storage. The storage bins were placed in wholesale refrigeration (1°C; 34°F). This storage was insulated, and provided a slow ice melting rate typical of most fish holds aboard vessels.



1. Fish in wholesale storage remained iced in the respective categories for two days prior to filleting and eight additional days for those not filleted.
2. The fillets, from whole fish stored 10 days following harvest, were stored eight days in retail.

Key: Whole Fish ; Fillets From Whole Fish

RR - Whole fish washed and iced with regular waters.
 RO - Whole fish initially washed and iced with ozonized waters at wholesale.
 OO - Whole fish initially washed and iced with ozonized waters in simulated vessel storage.

RRrr - Fillets washed and iced with regular waters after filleting RR fish.
 RRro - Fillets washed and iced with ozonized waters after filleting RR fish.
 ROoo - Fillets washed and iced with ozonized waters after filleting RO fish.
 OOoo - Fillets washed and iced with ozonized waters after filleting OO fish.

Figure 1. Schematic flow pattern of the experimental plan to evaluate variable applications of ozone washes and ice to preserve fish on the vessel and in wholesale, and for fillets in retail.

Prior to simulated vessel storage, the fish were spray washed 3 to 5 seconds and again reiced, this time with regular ice or with ice made from ozonized water to provide the whole fish categories shown in Figure 1. Whole fish receiving these treatments remained in wholesale refrigeration, as is customary for processors balancing inventory and demand. There were no additional washes or reicings, but whole fish were taken periodically for microbial analyses, sensory evaluations, and filleting.

After two days of wholesale storage, portions of the three wholesale fish categories were filleted (skinless), spray washed, and reiced to provide four additional categories of grouper fillets (Figure 1). These fillets then were delivered to a retail store, packaged to simulate current retail practice, and placed on an inclined bed of ice. Two layers of vinyl plastic film were used to prevent direct contact of the fillets with the ice. Each day, the fillets were rinsed and recovered with a sprinkling of ice to prevent dehydration. The model display cases were maintained at 35°F to 38°F (1.6°C to 3.3°C). The daily water rinses and sprinklings with ice employed regular or ozonized water/ice, depending upon the respective fillet categories. The regular and ozonized ice were supplied from the same source as used at the wholesale establishment.

Ozonized water for washes and ice was produced from the same municipal supply used for regular water washing and ice production. Ozonation systems were operated to maintain residual ozone concentrations of 0.3-1.0 mg/L, measured periodically with the DPD procedure. One ozone unit was installed at the wholesale/processing firm to provide water and ice for whole fish. A second unit was installed in the retail store for preparing ozonized water/ice for rinsing/storing fillets.

Microbiological Analyses--Periodically, whole fish and fillets were taken from each of the seven categories (Figure 1) to conduct aerobic plate counts (APCs) at 25°C, thio-

barbituric acid (TBA) analyses, and sensory evaluations. All microbial methods employed followed standard APC procedures /8/. Sections measuring 1.25 x 1.25 x 0.5 inch were removed from all whole fish and fillets using sterile templates, forceps, and scalpels, then weighed to the nearest 0.01 g. All sections weighed 25.0 ± 1.2 g. A section was removed from each whole fish dorsal side just posterior of the gill plate. With fillets, a similar section was removed, and also a second sample four inches posterior of the first section. On each sampling date, three whole fish and four fillets were analyzed per fish category. Each analysis was performed in replicate. Each weighed section then was homogenized in a sterile blending cup using 10:1 dilution by weight (approx. 250 mL) of 0.1% Peptone. Five serial dilutions (10^{-1} to 10^{-5}) then were made of the initial homogenizate using 0.1% Peptone. Each dilution (0.25 mL) was transferred using a sterile pipette to a Petri dish containing sterile aerobic plate count agar. A sterile "hockey puck" was used to spread the dilution. Each dilution was run in replicate, and blank controls were made at the beginning and end of each sampling run. Colony-forming units were counted after three days of incubation at 25°C and reported as the number of colonies/g wet weight of tissue. These counts then were averaged to obtain a mean count/g for each processing procedure used.

TBA Analyses /9/--Two analyses were conducted on each fish or fillet sample per sampling period.

Sensory Evaluations--Initially, raw fish and fillets were evaluated according to the experience judgements of the project investigators, recording an acceptance rating based on a 3-stage acceptance scale, in which A = Acceptable Quality with pleasant, fresh fish aroma; firm flesh with some translucence; clean; B = Acceptable Quality, but noticeable off-odors, some softening and clouding of flesh, and initial browning of the red muscle tissue; and C = Unacceptable Quality, with distinct putrid, off-odors, soft to mushy flesh, brown muscle tissues, and gaping.

On the same day, samples of cooked fillets were presented to a 6-member sensory panel, pretrained to rate cooked fillet appearance, aroma, texture, and flavor. Panelists were asked to rate acceptance relative to each sensory attribute and general overall acceptance. Panelists were prescreened to assure female members primarily responsible for food purchase and preparation for families. Each panelist was familiar with fish, served seafood at least once every 2 weeks, and had prior experience in sensory assessments of foods while employed with a local consumer opinion firm. Prior training sessions with fresh and near-spoiled grouper fillets helped acquaint the panelists and formulated the descriptive scale for each sensory attribute. On a scale of 1.0 to 5.0, the highest ratings were given fish with the highest acceptance -- those having ivory, white appearance, having bland, pleasant aromas and flavors, and firm texture. Lowest ratings of 1.0 were given to samples having low acceptance, appearing discolored, having off, fishy aromas and flavors, and having mushy textures.

The fillets were broiled to a standard internal temperature (75°C, 165°F), then were served as warm pieces for independent evaluations.

3. RESULTS AND DISCUSSION

Fish storage. After 2-3 days of actual vessel storage and 7 days of simulated vessel storage, the averaged microbial counts on regular iced and ozonized iced whole fish were essentially the same. Only after washing and reicing at the wholesale/processing facility was a bactericidal effect of ozone evidenced by the lower average APCs for 00 category fish. This difference persisted throughout the remaining 8 days of iced storage in wholesale refrigeration; however no differences were noted in fish appearance or odors to substantiate any obvious benefits from the lower microbial counts.

There was no apparent benefit from the intermittent application of ozone (R0) at the wholesale level. The acceptable ratings for all fish, regardless of water or ice exposure, diminished at a similar rate (Table I). After 17-18 days from the day of catch the raw fish were judged to be marginally acceptable. This shelflife is longer than that of various cold water fishes, but is common for similar groupers and snappers which are properly chilled and iced.

Fillet storage. Continuous storage in ozonized ice and daily washes with ozonized water retarded the total microbial growth on the fillets; however, this difference was not obvious until the third day of retail storage. Daily rinses with ozonized water may have provided some bactericidal effect, but comparing the gradual increase in microbial counts on RRrr and OOoo fillets suggests additional benefits from continual

applications of ozonized waters from vessel through retail. Continual applications retarded microbial growth and may have altered the bacterial flora. There were no apparent benefits from intermittent ozone applications (RRoo and ROoo).

TABLE I. SENSORY JUDGEMENTS* FOR THE WHOLE FISH CATEGORIES STORED IN ICE AT THE WHOLESALE/PROCESSING FIRM

Days After Harvest	Days** in Storage	Fish (in wholesale)			Fillets (in retail)			
		RR	RO	OO	RRrr	RRoo	ROoo	OOoo
11	1	A	A	A	A	A	A	A
13	3	A(B)	A(B)	A(B)	A(B)	A(B)	A(B)	A(B)
16	6	B	B	B	B(C)	B(C)	B(C)	B
18	8	B(C)	B(C)	B(C)	C	B(C)	B(C)	B(C)

* Ratings are based on a three-stage acceptance scale referenced during daily inspections

** Days in Storage represents the number of days the fish remained in wholesale storage and fillets remained in retail storage after the fish were caught 10 days prior, then filleted.

The most obvious changes in quality occurred between 3 and 6 days in retail storage. On the 6th day the fillets appeared softer and emitted some off-odors. These changes progressed to poor quality on the 8th day, but the fillets never exposed to ozonized water (RRrr) were the only fillets judged unacceptable. These fillets had the highest microbial counts, approaching 10^7 microorganisms/g.

Despite some differences in microbial counts and raw sensory judgements, the sensory panel evaluations for cooked fillets remained well above average for all fillet categories even after 8 days of retail storage. The averaged panel ratings could not detect unsavory attributes, particularly odors, as perceived by the investigator's judgements of the raw fillets.

The cooking process diminished odors noted in the raw fillets. The panelists remained pleased with the cooked fillets, even after 8 days of retail storage (after 18 days since harvest). These results did not distinguish any advantages for ozonation, but average flavor ratings were always highest for the fillets which had been exposed continuously to ozonized water/ice treatment since landing (OOoo). Flavor ratings generally agreed with the ratings for overall acceptance. There was no discernible pattern for comparison of ratings for cooked appearance, aroma, and texture.

The raw and cooked sensory evaluations for fish and fillets never detected any rancid odors or flavors. Likewise, the averaged TBA results ranged below levels commonly associated with development of rancid fish (Table II). These TBA values are lower than the same reported for numerous Gulf fish species, including grouper, stored at 1°C for 8 days /10/. Using the comparative TBA reference number of 1.0, the black grouper remained below the reference until the 10th day from harvest as compared to only 1 day for salmon and haddock /11/. Thus the presence of ozone in water washes and ice did not appear to enhance oxidative rancidity. This is not unexpected, realizing that the proximate composition of grouper flesh is only about 1.0% fat /12/.

4. COST/BENEFIT ANALYSES

For retail store applications, an ozone generating and application system capable of producing 2 to 10 g/h of ozone is an appropriate size, along with a water tank of 100 gallons. Relatively small quantities of ozonized water will be required for ice-making at this level compared with the volume of water required for washing fish morning and night. For smaller wholesaler/processors, a 25 g/h ozone system is appropriate, along with a 500 gallon water tank. Wash water demands of wholesaler/processors continue through the day, as opposed to the retailer. Ice-making requirements for ozone still are lower than demands for wash waters. Larger wholesalers will require a 50-g/h ozonation system and a 1,000 gallon tank. These ozonation systems all can be prefabricated so as to be plugged into a standard 110-V outlet, and plumbing hookup of a water inlet and outlet pipe.

Based on these size considerations, the current cost of appropriate ozone systems will be approximately \$15,000 for a 10 g/h system for retail stores, \$18,000 for a 20 g/h system for small and \$23,000 for a 50 g/h system for large wholesaler/processors. Daily operating costs per day, respectively, are 12 kWh (\$1.20 based on \$0.10/kWh), 25

kWh (\$2.50), and 40 kWh (\$4.00) for the three sizes of ozonation system. Annual operating and maintenance costs are an estimated 12 man-hours for all three systems.

TABLE II. TBA ANALYSES (mg MALONALDEHYDE/kg FLESH) FOR WHOLE FISH AND FILLETS FROM CATEGORIES TREATED WITH REGULAR OR OZONIZED WATER WASHES AND ICE

Storage (days)	Fish (in wholesale)		Filletts (in retail)	
	RR	OO	RRrr	OOoo
1	0.85 ± 0.19	0.67 ± 0.12	0.81 ± 0.20	0.51 ± 0.06
6	0.71 ± 0.18	1.06 ± 0.07	1.00 ± 0.05	0.75 ± 0.15
8			1.10 ± 0.34	0.54 ± 0.09

Initial storage time is after 2-3 days actual vessel storage, plus 7 days of simulated vessel storage, then 2 days of whole-sale storage prior to filleting. Each value is the average of six analyses from separate samples per day.

Cost-effectiveness. Information developed in consultation with a number of retailers of fresh fish regarding average sales indicates that large fresh fish counters can generate approximately \$17,000 per week in sales, while smaller retail counters can generate approximately \$10,000/week in sales. Because of variations in the sizes of retail stores, fixed overheads, amounts of customer traffic, and profit margin differences between individual stores and supermarket chains, it is very difficult to define an average, industry-wide profit level for fresh fish counters. Instead, for purposes of making estimates of payback times for ozonation equipment, we have assumed that whatever extension of fresh fish shelflife is obtained by the use of an ozone system, this will result in a 10% increase in sales as well as in profits.

For annual profit level ranges assumed for current fresh fish counters, the estimated payback times range from less than 11 months for high profit fish departments to just over two years for lower profit fish departments. Annual operating costs will increase \$438 (primarily increased electrical energy), plus \$120 in maintenance costs (12 man hours per year at an average \$10.00/h).

For estimating savings which might be obtained from less spoilage of retail fish due to prolonged storage life, we assume that a new retail store purchases 2,000 lbs of fresh fish/week at an average wholesale cost of \$2.00/lb. If 15% of this fish is wasted due to spoilage (without ozone), this means that of the \$4,000 spent on fresh fish per week, \$600 is lost in spoiled fish. If the incorporation of ozonation will reduce this amount of fish loss by 50%, this means that the retail store purchasing 2,000 lbs of fresh fish/week will lose only \$300/week, or will save \$15,600/yr.

On this basis, the \$15,000 investment for the retail store ozonation system will be paid back in just over 12 months. In actuality, the payback time will be less, because the \$300/week in fish which is not lost to spoilage will be sold at a profit, generating sales above those projected for the same volume of fish not subjected to ozone preservation treatment.

5. CONCLUSIONS

1) Under the storage conditions of this study, continuous applications of ozonized water and ice did retard microbial growth on whole fish and fillets. However, raw sensory evaluations failed to detect any obvious differences until the 8th day of retail storage (fillets) or 18 days after harvest (fish). This ca. 13% extension of fresh fish shelflife was less than 2 days, and distinguished poor versus unacceptable quality.

2) Despite these ratings, sensory evaluations for cooked fillet flavor were always higher for the fish treated continuously with ozonized water and ice made from ozonized water beginning shortly after harvest.

3) The intermittent introduction of ozonized water washes and ice did not yield the same benefits noted for continuous applications initiated at harvest.

4) Sensory evaluations and thiobarbituric acid analyses did not detect any signs of oxidative rancidity, regardless of the protocols followed for ozone treatment.

5) Ozonation cannot overcome or be a substitute for abusive handling of fish aboard a fishing vessel, in ice production, while processing, or during retail practice.

6) Costs for installing ozonation systems in retail stores currently selling fresh fish are estimated to be recoverable over a period ranging from <11 to 25 months, depending upon the current profitability of the fish counter/store, and assuming that

increases of shelflife and extension of sensory qualities will generate 10% increases in sales and profits.

7) For retail stores installing ozonation equipment along with a new fish counter and planning to purchase 2,000 pounds of fresh fish per week, the additional costs of the ozonation equipment also are recovered in about 12 months, assuming that the use of ozone will reduce the wastage normally anticipated due to spoilage of fish by 50%.

8) All results reported in this study should be qualified relative to the use of grouper, a lean fish species from a warm water region. Earlier investigations conducted with cold water species (salmon and squid) have indicated that shelflife extensions of 33% to 50% can be obtained by applying similar ozone treatment protocols.

The authors are grateful for the financial sponsorship provided by the Gulf and South Atlantic Fisheries Development Foundation, Inc., Tampa, Florida (USA).

REFERENCES

1. W.J. BLOGOSLAWSKI, 1982, "Ozonized Ice as a Preservative", OZONNEWS 10(1/2):9.
2. W. NELSON, 1982, "The Use of Ozonized Ice to Extend the Shelf Life of Fresh Alaskan Fish", report submitted to Alaska Dept. of Commerce and Economic Development, Office of Commercial Fisheries Development, Anchorage, AK (Oct.).
3. W.J. BLOGOSLAWSKI, V.G. AMPOLA, R.C. LUNDSTROM, E.M. RAVESI, B.E. TUHKUNEN, and R.W. VAN TWUYVER, 1983, "Effect of Ozonized Ice on Preservation of Squid (*Loligo pealei*)", presented at Intl. Ozone Association, Sixth Ozone World Congress, Washington, DC (May 1983); in Proc. Sixth World Ozone Congress, p. 44.
4. T. HARAGUCHI, U. SIMIDU and K. AISO, 1969, "Preservation Effect of Ozone on Fish", Bull. Japanese Soc. Sci. Fisheries 35(9):915-919.
5. W.S. OTWELL, N. BLAKE, D.E. SWEAT, R.L. MARSCHALK, J.W. FARQUHAR, and R.G. RICE, "Ozonized Water and Ice to Preserve Fresh Fish - Grouper (*Mycoteroperca bonaci*)", Final Report of Contract 25-09-54622/2110, Gulf and South Atlantic Fisheries Development Foundation, Inc., April 1985.
6. J.J. LICCIARDELLO, 1984, "Product Quality Chemistry", a Research Note included in the May-June 1984 Bimonthly Activity Report, Gloucester Laboratory, Northeast Fisheries Center, National Marine Fisheries Service.
7. B.J. DeWITT, III, V. MCCOY, B.L. HOLT, D.K. ELLIS, G. FINNE, and R. NICKELSON, II, 1984, "The Potential Use of Ozonated Ice for On-Board Storage of Gulf of Mexico Shrimp", Proc. Ninth Annual Tropical & Subtropical Fisheries Technol. Conf. of the Americas, Brownsville, TX, Jan. 9-12, pp. 269-279.
8. M.L. SPECK, Editor, 1976, Compendium of Methods for the Microbiological Examination of Goods (Washington, DC: American Public Health Assoc.), 701 pp.
9. E.W. TURNER, W.D. PAYNTER, E.J. MONTIE, M.W. BESSERT, G.M. STRUCK, and F.C. OLSON, 1954, "Use of the 2-Thiobarbituric Acid Reagent to Measure Rancidity in Frozen Pork", Food Technology (July), pp. 326-330.
10. V.T. MENDENHALL, 1972, "Oxidative Rancidity in Raw Fish Fillets Harvested from the Gulf of Mexico", J. Food Sci. 37:547-550.
11. C.W. SWEET, 1973, "Activity of Antioxidants in Fresh Fish", J. Food Sci. 38:1260-1261.
12. F.D. SIDWELL, 1981, "Chemical and Nutritional Composition of Finfishes, Whales, Crustaceans, Mollusks, and Their Products", U.S. Dept. of Commerce, NOAA Tech. Memo. NMFSF/SEC-11, 432 pp.

L'EAU ET LA GLACE OZONISEES POUR LA CONSERVATION DES POISSONS FRAIS : MEROU (*Mycoteroperca bonaci*)

RESUME : Dans les conditions d'entreposage de cette étude, les applications continues d'eau et de glace ozonisée retardent le développement microbien sur les poissons d'eau chaude entiers et les filets. Cependant, les évaluations sensorielles n'ont pas fait apparaître de différences sensibles jusqu'au huitième jour après l'entreposage en magasin de détail (filets) ou 18 jours après la pêche. Cette prolongation d'environ 13 % de la durée de conservation des poissons frais était inférieure à deux jours, et ne permettait que de passer d'une qualité inacceptable à une qualité médiocre. Malgré ces estimations, les évaluations du goût des filets cuits sont toujours bonnes pour les poissons traités constamment dès la pêche avec de l'eau ozonisée et de la glace fabriquée avec de l'eau ozonisée. Les lavages intermittents avec de l'eau ozonisée ne donnent pas les mêmes résultats qu'un lavage continu dès la pêche. Les évaluations sensorielles et les analyses de l'acide thiobarbiturique ne font apparaître aucun signe de rancissement en oxydation, indépendamment des protocoles suivis pour le traitement à l'ozone.

DISCUSSION

J.W. JEBSEN (Norway) - 1) Will ozone affect material used for electrical insulation and damage equipment in the fish processing factory ?

2) Since ozone is toxic may it also be a danger to workers in the factory ?

R.G. RICE - Chlorine is also a poisonous gas and yet it is used commercially. I, therefore, see no danger to workers provided that proper equipment is used and precautions are taken. I admit there may be a risk with electrical equipment but we have used ozonised ice in commercial premises for sometime now without any problems.

CHILLED STORAGE OF WET
FISH IN MODIFIED ATMOSPHERES

DR. L. W. BAHRIS

Nordsee, Deutsche Hochseefischerei GmbH, Bremerhaven (FRG)

Fresh, neutral foodstuffs like meat, milk and fish spoil very quick. Spoilage is generally due to the action of micro organisms. They change the appearance, smell and taste of foods, so the food is no longer suitable for human consumption. This, of course, is much more critical if micro organisms cause the food to become toxic.

Since the beginning of history mankind was keen to try to prolong the shelf-life of foods and to avoid spoilage. Cooling is very well known but this inhibits deterioration for only a few hours or in some case up to some days. Other well known methods change the nature of the products in such a way that they can no longer be compared to the fresh food. The methods are acidification, brining, addition of sugar, drying, smoking and some fermentations. More recently pasteurisation, sterilisation, freezing and deep freezing and chemical preservation have been developed.

Wet fish and fresh fish fillets are more sensitive to spoilage than fresh meat and in keepability are comparable to fresh milk. Micro organisms present in the natural environment of fishes, especially gram-negative micro organisms adapted to low temperatures, attack the meat of fish. These species commonly have high proteolytic activities with the consequence that ongoing spoilage is very quickly detected by sensory evaluation. This can be confirmed by microbiological and chemical analyses. Though there is no clear relationship between the number of proteolytic organisms and the spoilage as evaluated by sensory means, the total count and the number of specific organisms does give an indication of the extent of deterioration. Here chemical analyses give a more definitive answer. These analyses include total volatile basic nitrogen (TVBN), trimethylamine (TMA) and for some fishes, histamine, which is found as a protein decomposition product in tuna, mackerel, sardines and herring. With fat fishes rancidity may also be estimated chemically.

Several possibilities exist to decrease the unwelcome action of micro organisms or to inhibit it. The traditional procedure for wet fish is cooling in ice during transport, storage and sale. At this temperature (0 to 2 °C), a shelf-life of about 1 week is attained. According to the hygiene-regulations in Germany, sale of

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

unpacked fish is only allowed in special fish shops or special counters in super markets. A chemical preservation or other treatment of wet fish and fresh fish fillets is not allowed.

Deep freezing is a very good preservation method but has the disadvantage that the fish must be thawed before cooking. All other methods change the product properties and are not suitable for fresh fish.

We have tried to find a packaging method for fresh fish which allows a sufficient shelf-life at commercially attainable cooling temperatures. Possibilities are vacuum packaging or packaging in modified atmospheres. Both methods have been intensively investigated by us.

The vacuum pack principally inhibits the growth of aerobic organisms i. e. those that require oxygen for growth. A high proportion of the spoilage flora fall into this category. Facultatively anaerobic and strictly anaerobic organisms may grow in a vacuum pack. This means that vacuum packed wet fish must be stored at or below 2 °C. At higher temperatures the product will readily spoil and eventually there exists the risk that it may become toxic due to the growth of the anaerobic organism, Clostridium botulinum.

For this reason we decided to look for packages with protective aerobic atmospheres. It should however be said that a vacuum pack offers an excellent presentation form for fresh fish.

Protective atmospheres normally contain the main component gases of air in varying combinations. One has to distinguish between controlled atmospheres (CA) and modified atmospheres (MA). Controlled atmospheres have been used for the commercial storage of fruit and vegetables for a considerable time. Here the composition of the gas, which is usually enriched in carbon-dioxide (CO₂), is maintained constant. This is only possible in storage depots and not in closed packs. Modified atmospheres are used for single packs. During packaging the packs are first evacuated and then flushed with the gas mixture and sealed. When sealed a control and regulation of the composition of the gas mixture is naturally no longer possible. Because reaction between the product and the gas mixture may occur and because the packaging foils are not totally gastight so that there will be a certain exchange between the inner and outside atmospheres, the composition of the gas-mixture may change during storage.

We have done trials with different kinds of fish and used different compositions of gases mixtures. In every case we only used the main constituent gases of air:

nitrogen, oxygen and carbon-dioxide. The packs were prepared on a Multivac Form-Fill-Seal-machine, using, for the tray, a PVC/PE-foil, and for the top film a transparent PVDC-coated PVC-foil with antifog finish.

For collecting the drip loss we put an absorbent paper into the tray. The trials were carried out with air, as control, with pure nitrogen, with pure carbon-dioxide as well as with different mixtures of nitrogen, oxygen and carbon-dioxide. The samples were stored at 2, 4 and 8 °C and evaluated by sensory analyses (appearance, smell, taste and consistency) every 1 to 2 days. For tasting the fish was cooked in its own juice in a plastic bag. This method of preparation highlights changes in the taste and is relatively severe.

The results of these trials will be presented shortly. As compared to air, pure nitrogen does not increase the shelf-life. Nitrogen is inert, that means it does not react with the product and it has no influence on the growth of micro-organisms except of course to prevent the growth of strictly aerobic organisms. Pure carbon-dioxide has a very good inhibiting effect on micro-organisms but it has the disadvantage of dissolving in the tissue fluids. This results in a loss of pressure in the pack and the pack collapses. Furthermore, carbon-dioxide has the highest permeation rate of the 3 gases, and this will contribute to the collapse of the packs. With pure carbon-dioxide or high amounts of carbon-dioxide the fish becomes slightly acid and this is detectable by smell and by taste even after cooking. Finally, we selected a mixture with an amount of carbon-dioxide between 15 and 30 %, which we found most suitable for many different edible fish species. This mixture guarantees a good product quality and a shelf-life of 7 days at temperatures of 2 to 4 °C.

This modified atmosphere was suitable for cod, haddock, saith (coal fish), herring, trout, dover sole, plaice and salmon, but not for red fish (ocean perch). To date we have not been able to find a suitable gas mixture for protecting and maintaining the quality of red fish. For the successful packaging of fish in modified atmospheres, it is essential that raw materials be of excellent quality, the low temperature of the fish is maintained at all steps of manufacture, and rigorous hygienic conditions are employed in the factory. The maximum allowed temperature of the packaged wet fish during storage, transport, distribution and sale is 4 °C. If these requirements are fulfilled a shelf-life of 7 days can be obtained.

Packaging of wet fish does not prolong shelf-life but the product is then suitable for self-service and can be offered in all retail-food-shops.

RESUME : La conservation en magasin de détail de poisson frais entier ou de filets de poisson frais est d'environ une semaine lorsqu'ils sont entreposés dans de la glace à des températures de 0 à 2°C. La vente de poisson frais non emballé est cependant limitée aux poissonneries. Pour que ce poisson frais soit plus largement proposé au public, on a mis au point un nouveau système d'emballage utilisant une atmosphère modifiée. Les paquets peuvent être vendus dans tout magasin d'alimentation générale disposant de bonnes installations frigorifiques.

Du poisson de première qualité a été choisi pour l'essai. L'emballage était effectué avec une machine formant, remplissant et soudant. Juste avant la soudure, les emballages étaient mis sous vide et rincés avec un mélange de dioxyde de carbone-oxygène-azote. Les essais d'entreposage ont montré qu'on obtenait une durée de conservation commerciale de 7 jours à 2-4°C. CO₂ avait pour influence d'inhiber le développement d'un certain nombre de microorganismes responsables de la détérioration, ce qui permettait d'utiliser un entreposage à 4°C, au lieu de 2°C pour du poisson non emballé normal. Ce système d'emballage s'est révélé approprié pour le cabillaud, le lieu noir, l'églefin, la plie, la sole, la truite, le saumon et le hareng. Une commercialisation d'essai intensive est en cours.

COLD STORAGE OF SARDINES PACKAGED IN A MODIFIED ATMOSPHERE

G. BALDRATI, F. AMBROGGI and F. CASTELVETRI
Stazione Sperimentale per l'Industria delle Conserve Alimentari
Parma (Italy)

1. INTRODUCTION

Small pelagic species, particularly sardines (*Clupea pilchardus* W.) and anchovies (*Engraulis encrasicolus* L.), have a very important place in Italian fishery as they cover over one third of the whole national catch of fish (357, 129 t in the Italian in 1978). But, as often occurs when a plentiful resource and the correlated supply don't find an adequate demand, both these species, with the exception of fresh anchovies of a size suitable for salting, are inadequately utilized in our country. As regards sardines, apart from negligible amounts which are sold retail, they are either used as fresh or frozen raw material destined for further processing or exported or finally processed to meal. One of the main factors limiting the marketability of the fatty species is the difficulty of their preservation at low temperature.

In fact it is well known that, even if fish are properly frozen within a few hours from catching and then stored at -20°C , changes in the muscular tissue occur, adversely affecting their quality. Moreover the high seasonal variations in fat content of these species must be considered.

The shelf life of sardines, in particular, is limited by oxidative lipid changes which cause a rancid odor and discoloration of the flesh, and by severe protein myofibrillar changes which bring about significant deterioration of the product texture with loss of some functional properties of the muscular tissue. The latter depend on the freezing and the former on the storage at low temperature /1/.

For this reason we started some preliminary research in order to extend the shelf life of this kind of fish at low temperature.

2. EXPERIMENTAL

Raw material

Fresh sardines (having an average size of 25 fish/kg) caught in June 1984 in the northern Adriatic Sea were used. During the time elapsed from landing to use (about 12 h) the fish boxed in crushed ice were maintained at $+2^{\circ}\text{C}$ in a refrigerated store. The proximate composition of the sardine flesh was the following: dry matter 25.0%; protein 19.58%; oil 3.22%; ash 2.04%.

Packing in modified atmosphere

It was done by using, instead of plastics bags having a low permeability to the air, 1500 ml glass jars supplied with a twist-off gas-tight cap. Packing and conditioning with modified atmosphere was carried out on a special bench (connected with a carbon dioxide cylinder) placed inside a glove-box cabinet maintained under controlled atmosphere by gas pressure. To each jar were introduced 12-13 sardines chilled or frozen and glazed; after packing the containers were closed, taken out of the cabinet then stored at $+2^{\circ}\text{C}$ or -20°C .

Head space gas analysis

It was carried out by using a gas-chromatograph Dani 3200 equipped with a suitable device for gas drawing, a thermo-conductivity detector, a molecular sieve column, a Poropak T column and a valve to invert the succession order of the two columns to the flow of helium used as carrier. The gas composition of the conditioned jars resulted as follows: CO_2 91.9%; N_2 6.4%; O_2 1.7%.

Freezing of fish

It was carried out on a perforated belt conveyor of a pilot freezer with blown air circulation. The operative conditions were: unit load (kg/m^2 , h): 14; air speed (m/s): 5; freezing time (min): 20; initial temperature of the fish: $4-5^{\circ}\text{C}$; final temperature: -20°C . After freezing, sardines were glazed by dipping in cold water with ice.

I.I.F.- I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

Storage and sampling for analysis

The jars filled with chilled fish, in air or modified atmosphere, were kept in a cold store at + 2°C. Sampling for the microbiological and chemical analysis was carried out at 0, 2, 4 and 7 days of storage. The fish were beheaded, gutted and filleted and the fillets were minced and thoroughly mixed.

The jars filled with frozen and glazed fish, in air or modified atmosphere, were stored at -20°C + 1°C. In this case sampling for the chemical analysis was carried out at 0, 14, 46, 84, 111 and 152 days of storage. The fish, before being beheaded, gutted, filleted and minced were partially thawed by dipping in fresh running water.

Plate Count Agar

It was carried out on whole fish, suitably minced and diluted with physiological fluid solution containing 1 ‰ of peptone. Gram neg. bacteria were counted in Violet Red Bile Dextrose Agar (VRBG Institut Pasteur) while Gram pos. bacteria were counted in Plate Count Agar (PCA Difco) with addition of phenylethylalcohol (0.15%) to inhibit the Gram neg. organisms /2/.

Dry matter, ash and oil

According to official methods of A.O.A.C. /3/.

Total volatile nitrogen (T.V.N.)

According to Pearson /4/.

Crude protein

Total nitrogen was estimated by the method of Kjeldahl, using a Tecator automatic device, and per cent values were multiplied by 6.25.

Rancidity

Thiobarbituric acid method /5,6/.

pH

It was measured on minced fillets by a digital pH-meter Orsenigo mod. 440.

3. RESULTS

TABLE I
Storage at + 2°C

Storage days	pH		* T.V.N.		** TBA value		Gram + /g		Gram -/g	
	air	CO ₂	air	CO ₂	air	CO ₂	air	CO ₂	air	CO ₂
0	6.07		36		5.5		6.8x10 ³		1.8x10 ⁶	
2	5.98	5.94	46.2	63.0	21.3	12.3	4.0x10 ³	1.4x10 ⁴	6.2x10 ⁵	4.0x10 ⁵
4	6.05	6.10	61.6	98.0	29.1	13.5	5.2x10 ⁴	4.2x10 ⁴	6.0x10 ⁶	7.4x10 ⁵
7	6.18	6.13	66.6	47.6	42.8	11.8	1.6x10 ⁵	5.2x10 ⁴	5.0x10 ⁷	4.6x10 ⁵

0 = 24 h from the catch (iced fish)

* mg/100 g muscle

** mg malonaldehyde/kg muscle

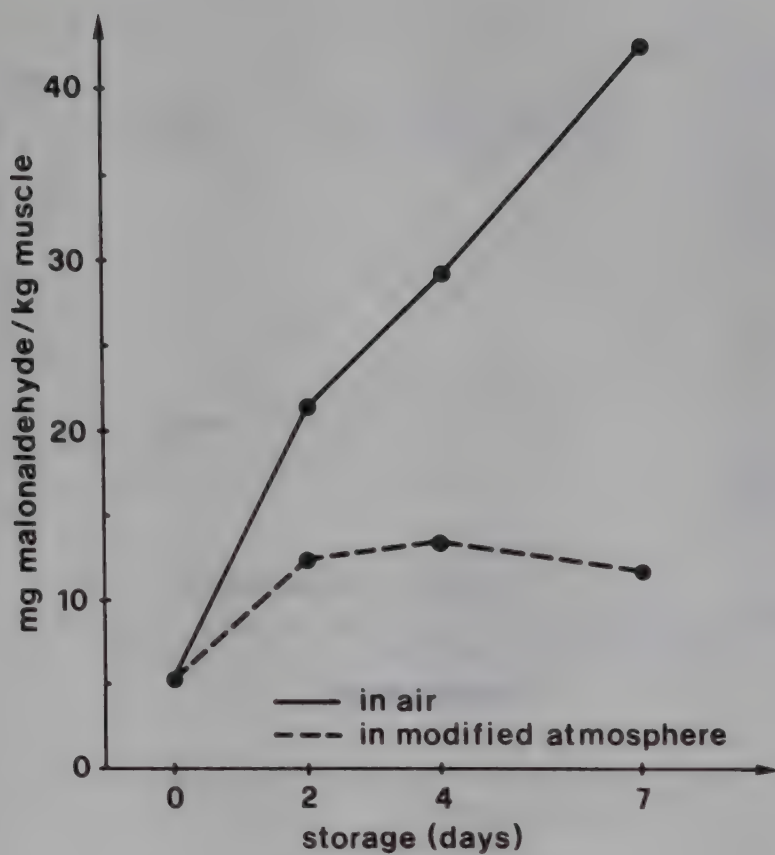


Fig. 1 - Effect of storage at 2°C on the rancidity of chilled sardines.

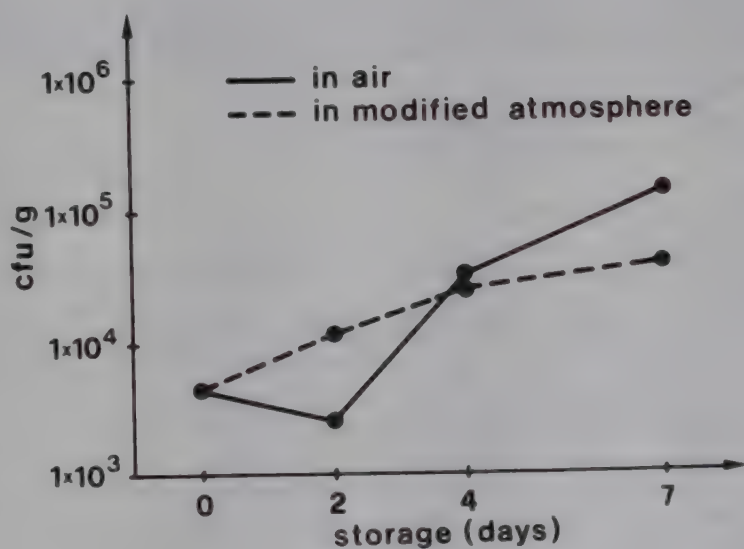


Fig. 2 - Effect of storage at 2°C on Gram pos. organisms.

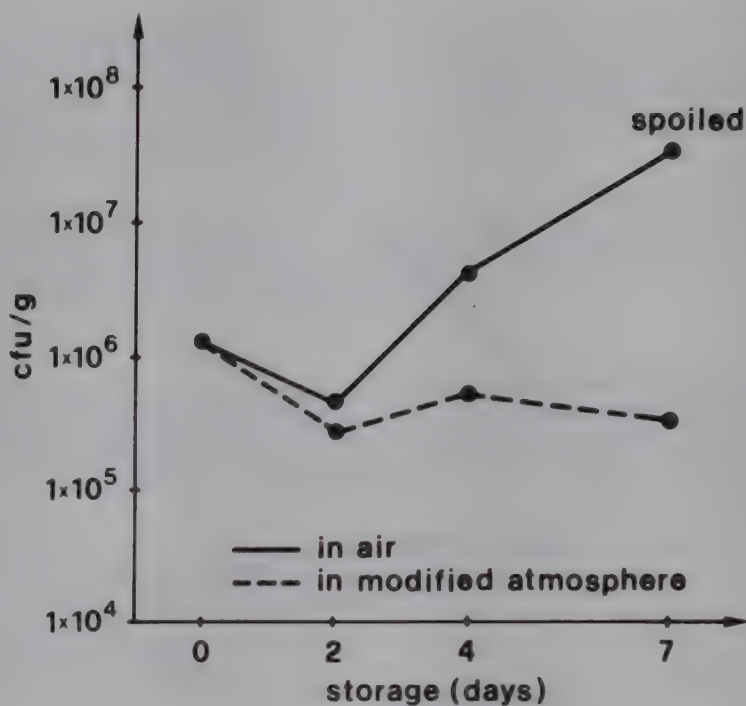


Fig. 3 - Effect of storage at 2°C on Gram neg. organisms.

From the data reported in table I and from fig. n° 1, 2 and 3, where TBA values, Gram neg. and Gram pos. plate counts are respectively plotted against storage time, it is clear that an atmosphere very high in CO₂, combined with a gas-tight package and a temperature of storage of 2°C, retards the spoilage of sardines and protects them against oil oxidation. Particularly the mode of action of CO₂ in delaying onset of spoilage in chilled fish seemed to be due, in agreement with the results reported from other authors /7, 8/, to the inhibition of psychrotrophic, aerobic, Gram neg. bacteria, even if TVN values were not correlated with microbiological evidence. Finally, as regards the storage tests of frozen and glazed fish, the effectiveness of a modified atmosphere in stabilizing the fish against oil oxidation may be inferred from table II and fig. 4.

TABLE II
Storage at - 20°C

Storage days	pH		* T.V.N.		** TBA value	
	air	CO ₂	air	CO ₂	air	CO ₂
0	6.07		36		5.5	
14	6.12	6.05	46.2	35.0	6.94	5.50
46	6.02	6.00	68.6	49.0	10.70	5.22
84	6.06	6.04	57.4	53.2	13.40	8.46
118	6.06	6.05	60.2	54.6	15.84	8.62
152	6.03	6.08	53.2	44.8	13.23	7.17

0 = 24 h from catch (iced fish)

* mg/100 g muscle

** mg malonaldehyde/kg muscle

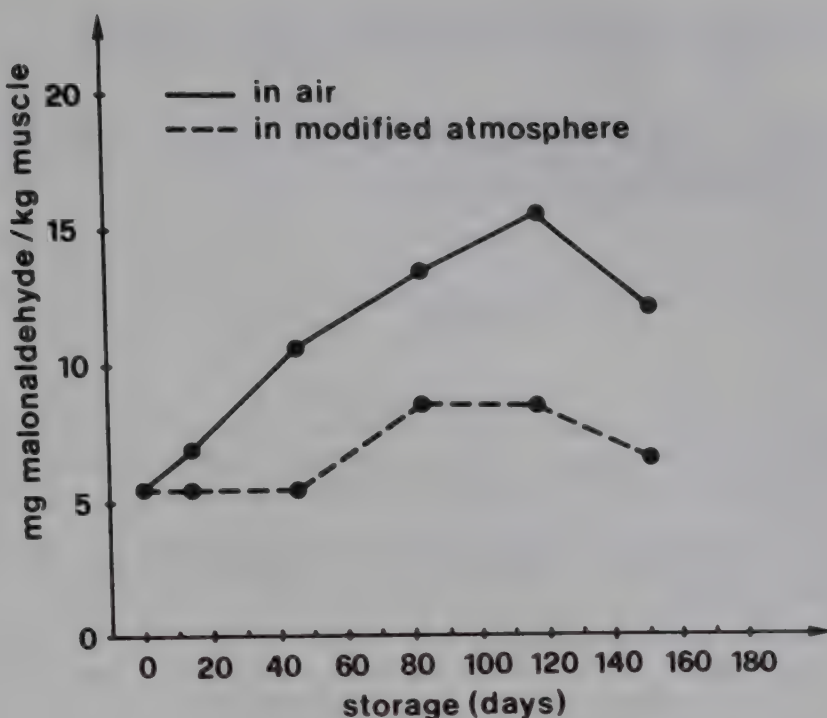


Fig. 4 - Effect of storage at -20°C on the rancidity of frozen-glazed sardines.

Briefly, this way of preserving the fatty fish can be considered very interesting even if, however, other gas mixtures with different percentages of carbon dioxide should be tested.

REFERENCES

1. G. BALDRATI, P. PIRAZZOLI, E. BROGLIA, F. AMBROGGI and I. INCERTI: Influenza del tipo di congelamento e del magazzinaggio a bassa temperatura sulla qualità delle sardine (*Clupea pilchardus* W.) [Effect of freezing method and cold storage on the quality of sardines (*Clupea pilchardus* W.)], Ind. Cons., Vol. 57 (1982) pp. 260-271.
2. B. D. LILLEY and J. H. BREWER, J. Am. Pharm. Assoc. Sci. Ed., Vol. 42, (1953) pp. 6-8 cited from M. R. W. BROWN, Inhibition and destruction of *Pseudomonas aeruginosa*, Inhibition and Destruction of the Microbial Cell, Edited by W. B. Hugo, Academic Press London - New York (1971), p. 342.
3. Official methods of analysis, Association of official analytical chemists, Washington, D. C., 1980.
4. D. PEARSON, Chemical analysis of foods, Butterworth, London, 1976.
5. B.G. TARLADGIS, B. M. WATTS, M. T. YOUNATHAN and L. DUGAN Jr., A distillation method for the quantitative determination of malonaldehyde in rancid foods, J. Am. Oil Chemists Soc., Vol. 37 (1960), pp. 44-48.
6. C.A.M. LIMA dos SANTOS, D. JAMES and F. TEUTSCHER, Guidelines for chilled fish storage experiments, FAO Fisheries Technical Paper n. 210, Rome 1981.
7. C. O. GILL and K. H. TAN, Effect of carbon dioxide on growth of meat spoilage bacteria, Appl. Environ. Microbiol., Vol. 39 (1980), pp. 317-319.
8. J. P. SUTHERLAND, J. J. PATTERSON, P. A. GIBBS and J. G. MURRAY, The effect of several gaseous environments on the multiplication of organisms isolated from vacuum - packaged beef, J. Food Technol., Vol. 12 (1977), pp. 249-255.

RESUME : Des recherches ont été effectuées pour évaluer l'action d'une atmosphère modifiée à teneur très élevée en CO_2 , combinée avec un emballage étanche et deux températures d'entreposage (2 et -20°C) sur la détérioration et sur l'oxydation de l'huile des sardines. On présente et discute les résultats obtenus.

DISCUSSION

M. HANUSAROTTIR (Faroe Islands) - The TVN values at the start are extremely high and there is a pronounced drop in the value for fish stored in a modified atmosphere between the 4th and 7th day of storage. Do you have any explanation for this and is it usual to have a high TVN content in cooled sardines ?

G. BALDRATI - There is no particular explanation for this although the fact that the sardines are ungutted may be partly responsible. The TVN values quoted in Table 1 are quite normal for sardines.

THE RELATIONSHIP OF TEMPERATURE TO THE SHELF LIFE OF
VACUUM PACKED MARINE FISH - AN INTERIM REPORT

MARGARET S. HENDRIE AND D.C. CANN

Ministry of Agriculture, Fisheries and Food, Torry Research Station,
135 Abbey Road, Aberdeen (United Kingdom)

With the introduction in the United Kingdom of legislation requiring the open dating of packaged foods /1/ it has become necessary to determine the shelf life of vacuum packed fish and fishery products. Experiments have been carried out to estimate the shelf life of marine fish species in vacuum skin packages (VSP) stored at a range of temperatures from 20°C to 0°C.

MATERIALS

Fish of the best quality obtainable was purchased locally and taken to the laboratory for filleting. In the case of mackerel it was necessary to purchase top quality fish in advance and freeze it until required. The species of fish used were cod, plaice and herring which were vacuum skin packaged as wet fillets and mackerel as hot smoked fillets. The fillets of the fresh or hot smoked fish were trimmed to a portion size of 100 g $\pm$ 10 g and packed in ice in insulated containers for overnight transport to the vacuum skin packaging facility. To prevent the fish becoming waterlogged from melt water, the ice was sealed in polythene bags. After packaging, the packs were replaced in ice in the insulated containers for the overnight return to the laboratory. Immediately on arrival, packs were assessed for quality prior to storage and the remainder were distributed to stores operating at 20, 15, 10, 5° and 1°C all $\pm$ 1°C. In the 1°C store the packs were kept in ice to give the definitive temperature of 0°C.

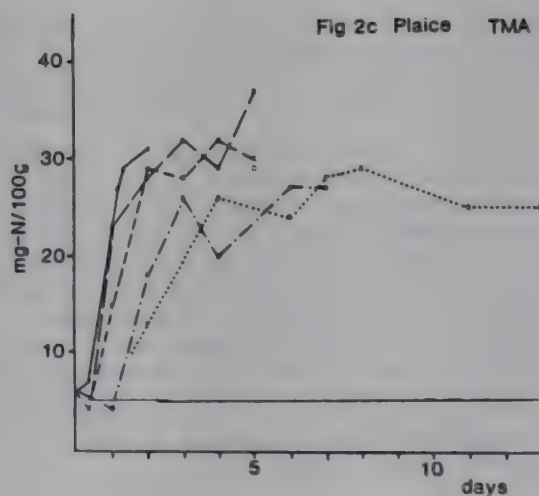
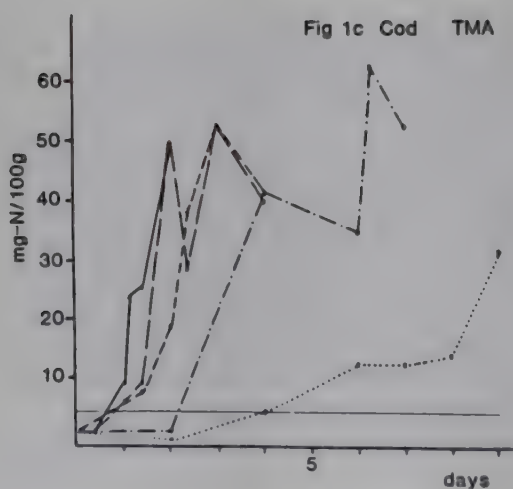
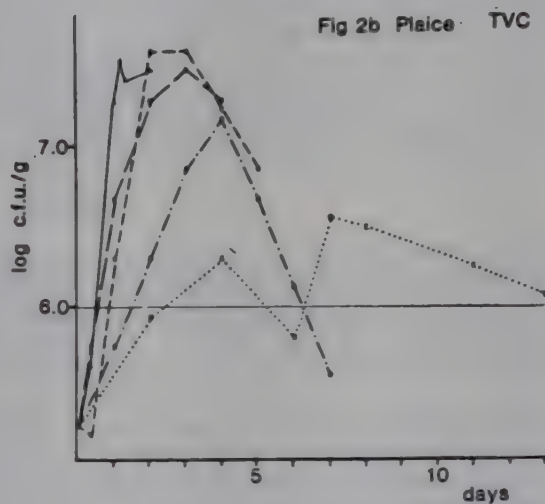
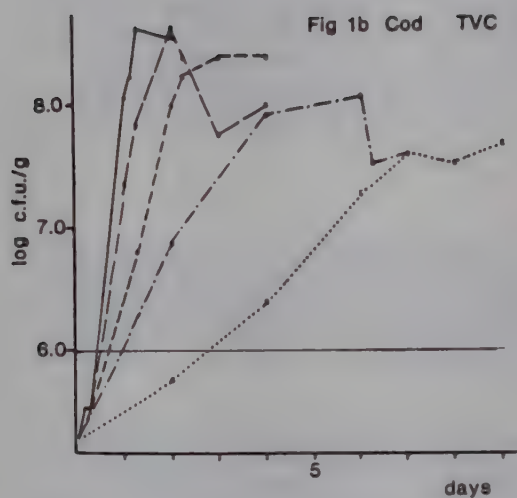
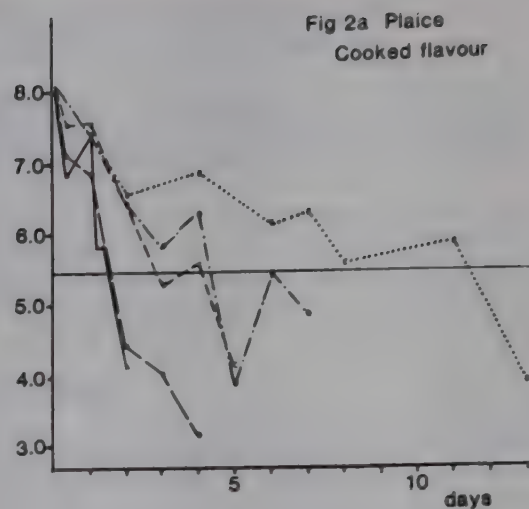
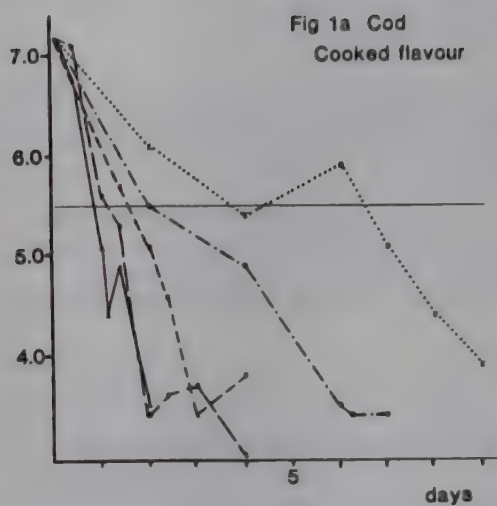
SAMPLING

To meet statistical requirements, in each experiment samples were analysed at suitable intervals to give a minimum of five sampling points for each storage temperature by the time that the fish were judged inedible by a trained taste panel. When a rejection level was reached, sampling of fish from that store was terminated provided that the minimum of five samplings had been taken.

At each sampling point 10 packs were taken for analysis - 5 for microbiology, 3 for chemical analysis and 2 for sensory assessment by the taste panel. The analytical methods used were those of Cann et al. /2/ except that the bacteriological investigations were limited to total viable counts (TVC) of aerobic bacteria at 20°C and 30°C and a count at 20°C of hydrogen sulphide-producing (H_2S -producing) bacteria.

RESULTS AND DISCUSSION

When defining shelf life of fishery products the quality requirements of a given market must be taken /2/ into account. For white fish packed under modified atmospheres, Cann et al. used the number of days taken to reach a cooked flavour score of 6.0 on the Torry scoring system when assessed by the trained taste panel, or for the total viable count at 20°C to reach 1×10^6 c.f.u./g fish, or for the level of trimethylamine (TMA) present in the fish to reach 5 mg-N/100 g fish. In this investigation the same levels of total viable count and TMA were used, but a taste panel cooked flavour score of 5.5 was taken as the end of acceptable shelf life. For herring and mackerel the end of acceptable shelf life as judged organoleptically was taken as a cooked flavour score of 3.0 on the Torry scoring system for herring and 5.5 on the Torry scoring system for mackerel. These scoring levels indicate a loss of good quality. Fish were judged to be inedible when the cooked flavour scores reached 4.5 for white fish, 4.0 for herring or 3.0 for mackerel. A total viable count at 20°C of 10^6 - 10^7 c.f.u./g or more was regarded as unsatisfactory as being above the maximum level suggested by the International Commission for Microbiological Standards for Foods (ICMSF) /3/



————— 20 C - - - - - 15 C - . - . - 10 C - - - - - 5 C 0 C

The results show that for cod there is reasonable agreement between the three indices of quality being considered - cooked flavour, total viable count at 20°C and TMA levels (Fig. 1a, 1b & 1c). The times to reach the limit for total viable count and TMA level are shorter than the time to reach the lowest acceptable cooked flavour score. The same pattern is seen in the results of the experiment with plaice (Fig. 2a, 2b & 2c). It is noteworthy that in this experiment the initial TMA level was just above the 5 mg-N/100 g limit (Fig. 2c) despite a high quality cooked flavour score of 8.0. However, there was no increase in the mean TMA levels after storage for 7 hours at 15°C and 10°C or 24 hours at 5°C, but overall the TMA levels increase in a manner similar to that of cod.

With herring and hot smoked mackerel there is a difference in the pattern between the three indices. A cooked flavour score of 3.0 on the herring scoring system (a numerically increasing score with loss of quality)(Fig. 3a) was reached before either the total viable count or the TMA level reached the acceptable limit (Fig. 3b & 3c). In 'fatty' fish there are changes in flavour due to factors other

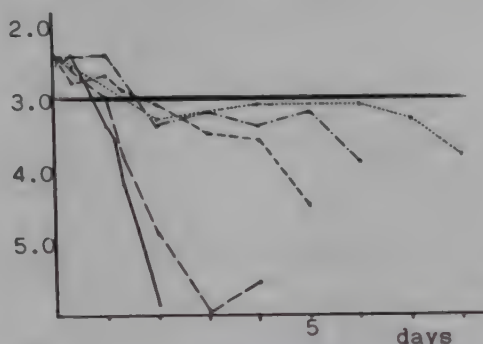


Fig. 3a - Herring Cooked flavour

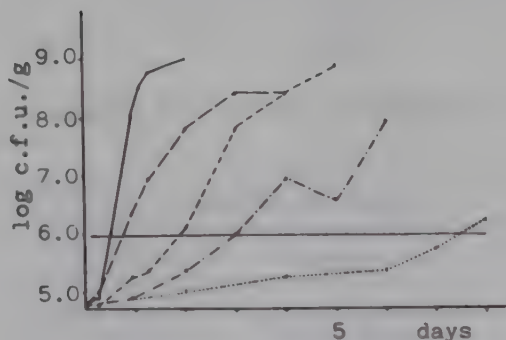


Fig. 3b - Herring TVC

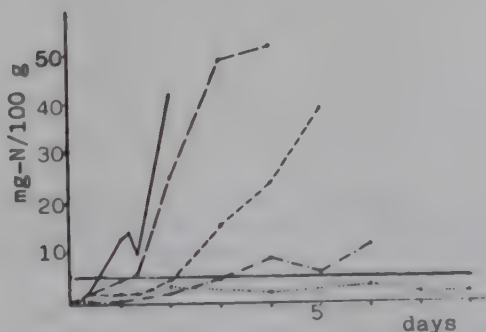


Fig. 3c - Herring TMA

than bacterial spoilage eg rancidity. Total viable count and TMA levels are less reliable indices of shelf life for herring, as the product may be rejected by the taste panel well before these indices have reached the limit of acceptability.

In the hot smoked mackerel experiment the lack of correlation between the taste panel cooked flavour scores and the total viable count and TMA levels was even more marked than with herring. Cooked flavour scores reached a level of 5.5 on the mackerel scoring system within 3 to 5 days storage at all temperatures (fig. 4). The total viable count on fish stored at 5°C and 0°C never reached a level of 10^6 c.f.u./g whilst at 10°C that level was reached only after 18 days storage (fig. 5). The level of TMA never reached 5 mg-N/100 g in packs stored at

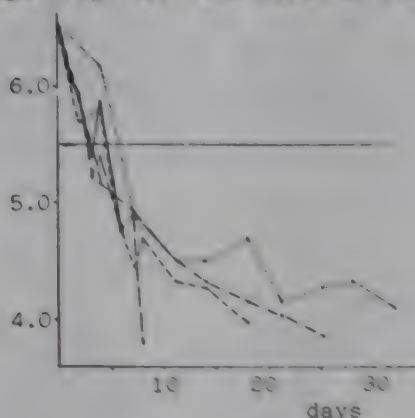


Fig. 4 - Hot smoked mackerel Cooked flavour

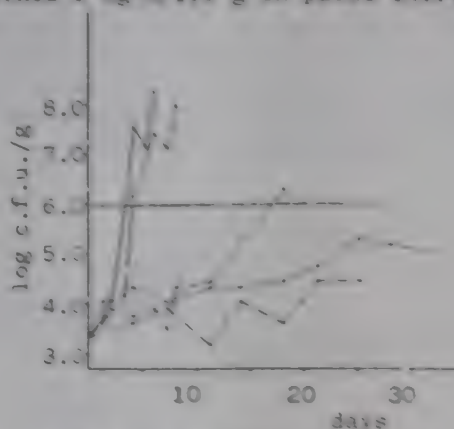


Fig. 5 - Hot smoked mackerel TVT

10°, 5° and 0°C even after 18, 25 and 32 days respectively. It was only at storage temperatures of 15° and 20° that there was a significant rise in total viable count and TMA level, but even then these indices were of little value in estimating the shelf life of the hot smoked fish. The salt, smoke and heat in the process all contribute to a reduction in the bacterial load present on the fresh fish and, in particular, affect the psychrotrophic bacteria important in the reduction of trimethylamine oxide in the fish to trimethylamine.

TABLE I

Calculated shelf life of VSF fish at various storage temperatures

Storage Temperature (°C)	Days to reach shelf life limit			
	Cod (Cooked flavour score 5.5)	Plaice (Cooked flavour score 5.5)	Herring (Cooked flavour score 3.0)	Hot smoked mackerel (Cooked flavour score 5.5)
20	3.9	1.5	0.5	3.7
15	1.2	1.75	0.5	3.2
10	1.5	3.4	1.6	3.5
5	2.6	4.6	2.5	4.0
0	4.9	9.1	3.1	5.7

At the end of shelf life, the product must be acceptable to the consumer, and should not have 'off' flavours when cooked. Date marking must allow sufficient time for distribution to retail outlets and consumption in the home. It can be seen from the regression calculations of the cooked flavour scores (Table I) that at storage temperatures of 10°C and over the shelf life of wet fish in vacuum skin packs is severely reduced and there is no margin for consumer abuse at temperatures of 15°C and above. At 5°C there is a significant extension of shelf life, which is further enhanced at 0°C, being extended about two fold for white fish but less so for

erring. Vacuum skin packed hot smoked mackerel was less affected by storage temperature, but again it was only at storage temperatures of 5°C and 0°C that significant extension of shelf life occurred. The relatively short shelf life of the smoked mackerel recorded here compared with earlier studies /2/ probably reflects the initial quality of the fish and also the interval between packaging the fish and commencement of the storage trial.

Clearly storage temperature has a marked effect on the shelf life of vacuum packed marine fish which is independent of other important factors such as initial quality of the fish. Application of the Olley and Ratkowsky /4,5,6/ equation for spoilage rates of wet fish at temperatures above 0°C to the present data shows that the bacteriological results give a better fit than the sensory results which gave an overestimate of about 25% in comparison with the theoretically calculated values (R.M. Storey, pers. comm).

ACKNOWLEDGEMENTS

The sensory evaluation was organised by Mrs A Craig. The chemical analyses were carried out by various chemists at Torry Research Station. G L Smith carried out the statistical analysis of the experimental data.

REFERENCES

1. Food Labelling Regulations, 1984. S.I. 1305 (84) London: H.M.S.O. Food Labelling (Scotland) Regulations, 1984. S.I. 1519 (S.128) London: H.M.S.O.
2. D.C. CANN, G.L. SMITH and N.C. HOUSTON : Further studies on marine fish stored under modified atmosphere packaging. Torry Research Station, (1983).
3. I.C.M.S.F. : "Microorganisms in Foods" 2. Sampling for Microbiological Analysis: Principles and Specific Applications. University of Toronto Press (1974) Chap. 8, pp. 92-104.
4. J. OLLEY and D.A. RATKOWSKY : The role of temperature function integration in the monitoring of fish spoilage. Food Technol. N.Z. Vol. 8 (1973) pp. 13-17.
5. J. OLLEY and D.A. RATKOWSKY : Temperature function integration and its importance in the storage and distribution of flesh foods above the freezing point. Food Technol. Aust. Vol. 25 (1973) pp. 66-73.
6. D.A. RATKOWSKY, J. OLLEY, T.A. McMEekin and A. BALL : Relationship between temperature and growth rate of bacterial cultures. J. Bact. Vol. 149 (1982) pp. 1-5.

RELATION ENTRE LA TEMPERATURE ET LA DUREE DE CONSERVATION DE POISSON EMBALLE SOUS VIDE. RAPPORT PRELIMINAIRE.

RESUME - La durée de conservation d'espèces de poisson de mer en emballage retreint sous vide dépend de la température d'entreposage, mais varie aussi selon les espèces et la qualité initiale du poisson avant emballage. Pour le poisson frais, la durée de conservation à des températures d'entreposage de 10°C et plus est courte, de 0,5 à 1,5 jours à 20°C, et passe à 1,5 et 3 jours à 10°C. Lorsque la température d'entreposage est abaissée, la durée de conservation est prolongée à 5°C et, de façon encore plus marquée par entreposage à 0°C. Pour le maquereau fumé à chaud, la durée de conservation est comparativement plus longue à des températures plus élevées, mais il y a là aussi augmentation de la durée de conservation lorsque les paquets sont entreposés à de plus basses températures.

DISCUSSION

H.H. HUSS(Denmark) - I question why the authors want to use TVC and level of TMA to determine shelf life. In my view there is absolutely no correlation between, for example, TVC and quality or even expected keeping time. This is particularly the case with smoked (and salted) fish. With respect to TMA, it is well known that the level of TMA is normally higher in vacuum packed compared to iced fish, consequently, the cut off point should be higher.

The results presented in the paper seems to prove my point.

M.S. HENDRIE - A number of features were measured to assess the quality of vacuum packed fish, of which three have been analysed in detail. - Total Viable Count (TVC) at 20°C, Trimethylamine level and Cooked Flavour Score. Total Viable Count is used by industry to assess quality. Thus it was measured so that the information was available. In wet white fish there is a correlation between total viable count and Trimethylamine levels.

We recognise that the end of shelf life will ultimately be determined by loss of flavour and other eating qualities as judged by the taste panel (or consumers). Our results underline the fact that there is little or no relation between the consumers acceptability of hot smoked mackerel (or other smoked fish) and total viable count.

R. BENNETT (U.K.) - Could you clarify what you mean by the term 'cooked flavour score' ?

My confusion arose because I thought you would present hot smoked mackerel samples without cooking to sensory panels.

M.S. HENDRIE - The cooked flavour of fish, steamed without condiments, is judged against descriptions of the flavour from very fresh, typical of the species when freshly caught, through loss of flavour to neutral or no flavour than to the detection of "off" flavours to strong sour and bitter flavours typical of spoiled fish. There is a different scoring sheet for different types of fish.

The hot smoked mackerel was tasted at room temperature without further cooking and the flavours scored against the system for cooked mackerel, (not smoked).

M. JUL (Denmark) - We just heard that "Nordsee" gave up vacuum packaging of fresh fish because of an assumed botulism hazard. We are aware of the enormous concern in the USA by the FDA regarding vacuum packaged smoked fish. You have made large scale experiments at Torry with these kinds of products. Would you care to give us your views on this subject of botulism hazard.

D.C. CANN and G. HOBBS (U.K.) - The potential hazard from Clostridium botulinum lies in possible situations where toxin could arise before the product is rejected on sensory grounds.

With fresh fish this can only arise under conditions of gross temperature abuse and since vacuum packaging has a relatively small effect on shelf life the hazard is no greater. In addition, this type of product would normally be cooked as an additional safeguard.

With smoked fish, control of C. botulinum is achieved by a combination of chilling and increased salt, but frequently there is not the additional safeguard of cooking. C. botulinum is inhibited by 3.0% salt (in the water phase) at storage temperatures up to 10°C. At higher temperatures toxin can arise before the product is rejected. Vacuum packaging gives a somewhat longer shelf life extension than with fresh fish and this could introduce an increased hazard. Under conditions of abuse (too high a temperature for too long a period) toxin may arise before sensory rejection in all forms of packaged smoked fish. It may also develop somewhat earlier in vacuum packed than in air packed products under equivalent conditions of storage.

VACUUM-PACKAGING OF FRESH FISH: EFFECT OF OXYGEN PERMEABILITY OF PACKAGING MATERIAL ON THE DEVELOPMENT OF BOTULINAL TOXIN

S.GOLA

Stazione Sperimentale per l'Industria delle Conserve Alimentari
Parma (Italy)

M.Rossi

Food Technologist, Packaging Technical Center, Cryovac
Division of W.R. Grace, Passirana di Rho (MI)(Italy)

SUMMARY: The present study was undertaken to evaluate the effect on botulinal toxin development of oxygen permeability of packaging materials used for vacuum-packaging of fresh fish.

To this aim hake fillets, inoculated at low (10^2 /g) and high (10^5 /g) levels with spores and vegetative cells of 5 *C.botulinum* strains (A, B, E types) were vacuum-packaged in materials with high and low oxygen permeability, stored at constant +10°C temperature or submitted to temperature abuse of +18°C during chilled storage, and evaluated for toxin development. Non-inoculated reference samples were kept for organoleptic and microbiological evaluations.

Organoleptic spoilage took place before the onset of toxin development, since the former occurred after 3-5 days at 10°C and after 24 h at 18°C, and the latter after 14 days at 10°C and after 4 days at 18°C. Oxygen permeability of packaging materials had a marginal effect on toxin development, which depended mainly on storage conditions and inoculum level.

CONDITIONNEMENT SOUS VIDE DE POISSON FRAIS; EFFET DE LA PERMEABILITE A L'OXYGENE DES MATERIAUX DE CONDITIONNEMENT SUR LA PRODUCTION DE LA TOXINE BOTULINIQUE.

RESUME: Cette étude sur le développement de la toxine botulinique a été conduite pour évaluer l'effet de la perméabilité à l'oxygène des matériaux de conditionnement utilisés pour le conditionnement sous vide du poisson frais. .

Dans ce but des filets de merlu inoculés à niveaux bas (10^2 /g) et élevés (10^5 /g) avec des spores et des cellules végétatives de cinq souches de *C. botulinum* (types A, B, E), ont été conditionnés "sous vide" en matériels d'emballage à haute et à basse perméabilité à l'oxygène: a) entreposés à 10°C. b) soumis à un excès de température de 18°C pendant la entreposage à l'état réfrigéré, et évalués pour le dépistage des toxines. Des échantillons témoins non-inoculés ont été conservés pour l'évaluation organoleptique et microbiologique.

Les résultats ont démontré que: - l'altération organoleptique précédait le développement des toxines, puisque les caractéristiques organoleptiques étaient déjà altérées après 3-5 jours à +10°C et après 24 heures à 18°C tandis que le développement de la toxine commençait après 14 jours à 10°C et après 4 jours à 18°C.

Les matériaux d'emballage perméable à l'oxygène avait un effet marginal sur le développement de la toxine, ce dernier dépendent des conditions de conservation et du niveau d'inoculation.

1. INTRODUCTION

Several growth and toxin production tests were performed by some Authors on fish packaged in vacuo and under modified atmosphere/1/2/3/4/5/6/, as a function of type of fish (cod and haddock fillets, herring, smoked salmon, trout), type and levels of *C.botulinum* and storage conditions. Some outbreaks related to the consumption of fish products have been reported by the Center for Disease Control/7/.

The introduction of fish pre-packaging methods could be a cause of potential health hazard due to botulism, because the micro-environment around the fish could be modified by fish pre-packaging methods.

This study was carried out in order to investigate the effect on botulinal toxin development of packaging material oxygen permeability used for fresh fish vacuum-packaging, relative to different types and levels of inoculum and different storage conditions.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

2. MATERIALS AND METHODS

Fish source. Fresh hake (*Merluccius merluccius*) was manually beheaded, gutted and filleted. Prior to inoculation, fillets were rinsed, drained and divided into portions of 100 g each.

Packaging material. Three packaging materials having different permeability were used in this study: O₂ permeability (at 23°C and 0%R.H.)

	(cc/M ² /24 hours/bar)
- Darfresh ⁽⁺⁾ film	< 1
- Cryovac BB ₁ ⁽⁺⁾ bag	20 - 40
- Cryovac ⁽⁺⁾ Super L bag	3000 - 4000

The pouches (15x25 cm, triplicate samples in each packaging material were prepared for each check time) were vacuumized and sealed but not shrunk in order to avoid any thermal shock and variation in permeability due to a change of the film thickness.

C.botulinum inoculum. The following five *Clostridium botulinum* strains were employed in this study:

- 62 A type A; ATCC 7949 type B; ATCC 25765 type B (non-proteolytic); ATCC 17786 type E; ATCC 17854 type E.

The fish fillets were inoculated (surface and inside of the flesh) with a suspension of spores and vegetative cells in order to obtain a final concentration of 10² and 10⁵/g.

Mouse assay for toxin. Each sample was macerated with physiological saline and centrifuged for 10 min. at 6000 rpm/min. The supernatant was incubated for 1 hour at 37°C with an equal volume of phosphate buffer (pH 6.2) containing 1% of trypsin (1.250, Difco).

Each of 2 or 4 mice was injected intraperitoneally with 0.5 ml of this mixture and observed for symptoms of botulism for a period of two days. Another batch of mice was protected with antisera (type A, B, E) and also inoculated with the same sample.

Microbiological analysis. Aerobic plate count was evaluated on Plate Count Agar (Oxoid) incubated at 18°C for 4 days. Sulphite-reducing clostridia were evaluated on M₅ medium/8/incubated at 30°C for 4 days.

Organoleptic evaluations. Fish odor was evaluated at the opening of the packages then after some minutes (10') of air exposure and finally after cooking (fish fillets were wrapped in aluminium foil and cooked for 10 minutes in boiling water). To express a judgement on organoleptic properties, score sheets prepared at Torry Research Station/9/ were used.

Test outline. In the first test the hake fillets were inoculated with 10⁵ and 10² spores and vegetative cells per gram, then vacuum-packaged in the three types of pouches and incubated at 10°C. In the inoculated and non-inoculated samples, microbiological, toxicological, and organoleptic analyses were carried out after 3,5,7,10,12,14 and 17 days.

In the second test the hake fillets were inoculated as before and subjected to two storage cycles: 1) a part was kept at a constant temperature of +2°C for 3,5,7,10,14 days, 2) the other part was incubated for three days at +2°C and then brought to 18°C for the following times: 12,24,48 hours and 4,7,9 days.

3. RESULTS

In preliminary tests hake fillets were inoculated with different (A,B,E) types and levels (from 10⁻¹ to 10⁵ spores and vegetative cells/g) of *C.botulinum* and vacuum-packaged in Darfresh pouches. The two batches of the samples were incubated at 4 and 8°C respectively: no botulinal toxin production was detected after 42 (4°C) and 8 days (8°C) respectively. In the samples containing the highest *C.botulinum* inoculum level and incubated at 8°C, type B toxin was found after 15 days.

First test. Aerobic plate count at time 0 was 5.2×10^6 cfu/g. After three days of storage at 10°C non-inoculated reference samples were microbially altered: 1.1×10^8 and 1.6×10^7 cfu/g were the aerobic-count values in the fillets packaged in Cryovac Super L and in Darfresh pouches respectively (Table I). After 7 days the fillets were altered; the sulphite-reducing clostridia that, at time 0 were present at low levels ($< 20/\text{g}$), increased ($\sim 10^6/\text{g}$) after 12-14 days'storage at 10°C .

Table I

Variation of aerobic plate count as a function of storage time at 10°C , in hake fillets.

Type of package	Storage time(days) /cfu/g			
	0	3	5	7
Darfresh [®] film	5.2×10^6	1.6×10^7	8×10^7	2×10^8
Cryovac BB ₁ [†]	"	1.2×10^7	4×10^7	1.2×10^8
Cryovac [®] Super L	"	1.1×10^8	4.4×10^8	1.4×10^9

Organoleptic properties after cooking were the most suitable and sensitive indicators of freshness. In fact, after exposure to air, odor of fillets stored for 3 days at 10°C was acceptable and better in gas-tight materials (Darfresh and Cryovac BB₁ bag) than in the permeable one (Cryovac Super L). The quality of cooked fish, however, was unacceptable because of the development of ammonia vapors from the flesh, especially in fillets packaged in gas-tight material. After seven days'storage, organoleptic properties were not tasted any more because the fish was too much spoiled.

As for toxin development, toxin was never found up to 12 days'storage in the inoculated samples and up to 17 days in non-inoculated ones. Type E toxin was found after 14 days of storage only in the samples inoculated with the highest level in all three packaging materials (Tab.II); after 17 days'storage the samples inoculated with the lowest level, vacuum-packaged in Darfresh film and BB₁ bag, proved to be toxic, whereas the samples kept in Super L bags didn't. The toxin detected was only of type E.

Similar results were reported by some Authors (Cann/2/, Eklund/10/, Ando/11/, Hussain/12/..

Table II

Botulinal toxin in inoculated hake fillets vacuum-packaged in Darfresh film, Cryovac BB₁, Cryovac Super L pouches, stored at $+10^\circ\text{C}$.
n.d. = not detected

Check-time(days)		Inoculum level(<i>C.botulinum</i> /g)	
		10^2	10^5
Type of package		Toxin	
3,5,7, 10,12	Darfresh [®] film	n.d.	n.d.
	Cryovac BB ₁ [†]	"	"
	Cryovac [®] Super L	"	"
14	Darfresh [®] film	n.d.	Present: type E
	Cryovac BB ₁ [†]	"	"
	Cryovac [®] Super L	"	"
17	Darfresh [®] film	Present: type E	Present: type E
	Cryovac BB ₁ [†]	"	"
	Cryovac [®] Super L	n.d.	"

Second test. a) After three days'storage uninoculated hake fillets packaged in the three types of packaging material were not altered microbially. Spoilage of fish occurred after 5 (1.4×10^7 cfu/g) and 14 (9×10^6 cfu/g) days in oxygen permeable (Cryovac Super L) and in gas-tight packaging material (Darfresh), respectively. Hake fillets were fresh and had no odours, after 3 days'storage at 2°C .

After 5 days of storage the organoleptic properties reached the limit of non-acceptability (according to Shewan's score) /9/ independently of the type of packing material : after cooking ammonia and soapy odors were especially in the fillets packaged in Cryovac Super L pouches. Organoleptic properties after cooking indicated freshness more than did microbial evaluations.

b) An interruption of cold storage for 12 hours was not sufficient to make fillets packaged in gas-tight material unacceptable from the organoleptic viewpoint, even if light ammonia traces were detectable in the flesh after cooking. On the contrary, the fillets packaged in Cryovac Super L bag were already spoiled with sweetish odors from raw fillets and soapy ammonia odors after cooking. Aerobic plate count varied from 9×10^6 cfu/g to 6×10^5 cfu/g in the fillets stored in Cryovac Super L and in Darfresh pouches, respectively.

An interruption for 24 hours of the cold chain was sufficient to determine the spoilage of the fish in oxygen permeable packaging material, only; the fish packaged in the other two materials was not microbially altered, but unacceptable from the organoleptic viewpoint.

c) Toxin development was never detected on inoculated samples packaged in gas-tight and oxygen-permeable materials, subjected to interruption (for 12, 24 and 48 hours at 18°C) of chill storage ($+2^\circ\text{C}$).

The inoculated samples with higher levels of *C. botulinum*, stored at 18°C for 4 days proved to be toxic (Type E) independently of packaging material oxygen permeability. Seven days' storage at 18°C caused type E production in all tested packaging materials, at both high and low levels of inoculum (Tab.III); Botulinal toxin was not detected after 9 days' storage at 18°C in uninoculated samples.

Inoculated samples were always unacceptable and spoiled prior to toxin detection.

Table III

Botulinal toxin in inoculated hake fillets vacuum-packaged in Darfresh film, Cryovac BB₁, Cryovac Super L pouches, stored at $+18^\circ\text{C}$.

Check-time(hours or days)		Inoculum level(<i>C.botulinum</i> /g)	
		10 ²	10 ⁵
Type of package		Toxin	
12 , 24 , 48 h	Darfresh [†] film	n.d.	n.d.
	Cryovac BB ₁ [†]	"	"
	Cryovac [†] Super L	"	"
4 days	Darfresh [†] film	n.d.	Present; type E
	Cryovac BB ₁ [†]	"	"
	Cryovac [†] Super L	"	"
7 , 9 days	Darfresh [†] film	Present; type E	Present; type E
	Cryovac BB ₁ [†]	"	"
	Cryovac [†] Super L	"	"

n.d. = not detected

ACKNOWLEDGMENTS

We thank Mr. D.C. CANN - Torry Research Station (Aberdeen) - for his invaluable suggestions, and Dr. P. PIRAZZOLI and Dr. G. ATTOLINI for their precious collaboration.

(+) Darfresh, Cryovac BB₁ and Cryovac are registered trade marks by W.R. GRACE.

REFERENCES

1. D.C. CANN, B.B. WILSON, J.M. SHEWAN, J. appl. Bact. 29 (3), 540, (1966).
2. D.C. CANN, B.B. WILSON, G. HOBBS, J.M. SHEWAN, J. appl. Bact., 28 (3), 431, (1965).
3. D.C. CANN, L.Y. TAYLOR, J. Food Technol., 14, 123, (1979).
4. D.C. CANN, L.Y. TAYLOR, J.M. COLLETT, Proc. of the world congress Food borne infections and intoxications. Berlin, p.826, (1980).
5. D.A. KAUTTER, J. Food Sci., 29, 843, (1964).
6. M.W. EKLUND, Food Technol., 36 (XII), 107, (1982).
7. Center for Disease Control. Botulinum in the United States 1899-1977. Handbook for epidemiologists, clinicians and laboratory workers. Atlanta GA. US Department of Health. Education and Welfare. Public health Service. (1979).
8. A. CASOLARI, Proc. 4th Int. Cong. Food Sci. Technol., Madrid 3, 86, (1974)
9. J.M. SHEWAN, R.G. MACINTOSH, C.G. TUCKER, A.S.C. EHRENBURG. The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice; J. of Sci. Food Agric., 4 (6), 294, (1953).
10. M.W. EKLUND, F.T. POYSKY, The significance of non proteolytic C. botulinum types B, E, F in the development of radiation-pasteurised fishery products. Preservation of fish by radiation. Vienna, Atomic Energy Agency 125, (1970).
11. Y. ANDO, T. KARASHIMADA, Food Irrad. (Shokuhin-Shosha) 8, 118, (1973).
12. A.M. HUSSAIN, D. EHELMANN, J. F. DIEHL, Arch. Lebensmittelhyg., 28, 23 (1977).

CONDITIONNEMENT SOUS VIDE DE POISSON FRAIS ; EFFET DE LA PERMEABILITE A L'OXYGENE DES MATERIAUX DE CONDITIONNEMENT SUR LA PRODUCTION DE LA TOXINE BOTULINIQUE.

RESUME - Cette étude sur le développement de la toxine botulinique a été effectuée pour évaluer l'effet de la perméabilité à l'oxygène des matériaux d'emballage utilisés pour le conditionnement sous vide du poisson frais.

Dans ce but, des filets de merlu inoculés à des niveaux bas (10^2 /g) et élevés (10^5 /g) avec des spores et des cellules végétatives de cinq souches de C. botulinum (types A, B, E), ont été conditionnés sous vide dans des matériaux d'emballage à grande et à faible perméabilité à l'oxygène :

a/ entreposés à 10°C,

b/ soumis à une température excessive de 18°C pendant l'entreposage à l'état réfrigéré, et évalués en vue de la détection des toxines.

Des échantillons témoins non-inoculés ont été conservés pour l'évaluation organoleptique et microbiologique.

Les résultats ont montré que : l'altération organoleptique précédait le développement des toxines, puisque les caractéristiques organoleptiques étaient déjà altérées au bout de 3 à 5 jours à +10°C et au bout de 24 heures à 18°C, alors que le développement de la toxine commençait au bout de 14 jours à 10°C et au bout de 4 jours à 18°C.

Les matériaux d'emballage perméables à l'oxygène avaient un effet marginal sur le développement de la toxine, ce dernier dépendant des conditions de conservation et du niveau d'inoculation.

SECTION VI

ETUDES SUR L'ENTREPOSAGE DES POISSONS
ET AUTRES PRODUITS DE LA MER RÉFRIGÉRÉS

*CHILLED STORAGE STUDIES ON FISH
AND SHELLFISH*

STORAGE LIFE OF CHILLED AFRICAN FISH SPECIES

M. MLAY

Nyegezi Fisheries Training Institute
Mwanza (Tanzania)

N. DIOUF

Institut Technologique Alimentaire
P.O. Box 2765
Dakar (Senegal)

Z. KARNICKI, C. LIMA DOS SANTOS
Fishery Industry Division
Food and Agriculture Organization
Rome (Italy)

1. INTRODUCTION

Considerable amounts of data on storage life of chilled fish from cold or temperate waters are available, Bramsnaes (1). Much less is known about storage life of chilled tropical species, Lima dos Santos (2), and very little about storage life of chilled tropical fish handled in commercial conditions. Many fish handlers or processors in the tropics, particularly in Africa are not very much aware about the advantages of using ice and possibilities of prolonged storage life. Inappropriate and insufficient use of ice together with lack of proper insulation of stored fish gives a false picture about the usefulness of icing as a preservation method and the advantages which may derive from it.

Aiming to demonstrate and propagate proper icing and handling of iced fish, FAO, within its Cooperative Research Programme on Fish Technology in Africa, launched a storage life study of several commercially important fish species in Africa. Experiments were carried out in Senegal at the Food Research Institute in Dakar and Tanzania, at Nyegezi Fisheries Training Institute in Mwanza. Practical demonstration of prolonged storage life of fish kept in ice or chilled water and its superior quality led in Senegal to construction and introduction of large 1.3 t/capacity insulated containers for handling of fish on board pirogues. Construction of these containers has been described in FAO Circular No. FIIU C/775.

2. MATERIAL

Storage life studies have been carried out on both, fresh and marine water species. Freshwater species were represented by (*Burjurkoma* sp.), lungfish (*Protopterus ethiopicus*) and Nile perch (*Lates niloticus* spp.). Marine species used in experiments were sardinella (*Sardinella aurita*) and seabream (*Epallus coqui*).

All samples were caught by local boats and preserved in three different ways:

Lot A - Without chilling, in ambient temperature of 25°C in case of freshwater species, and 20-30°C in case of marine species.

Lot B - Stored in plastic fish boxes, well iced.

Lot C - Stored in chilled lake or sea water (CLW/CSW) in insulated 200 l capacity fish containers at 0°C.

All species, except Nile perch were stored whole, ungutted. Nile perch due to its large size was filleted and fillets were packed tightly in plastic bags stored in accordance with above-mentioned conditions.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

3. METHODS

3.1 Sensory Assessment

Sensory assessment of cooked samples were carried out using a 10 point hedonic scale, L. dos Santos *et al.*, (3).

3.2 Total Volatile Bases (TVB)

Total Volatile Bases were estimated using the Conway microdiffusion method.

3.3 Microbiological Analysis (TPC)

Total plate count was estimated as described by Lima dos Santos *et al.* (3).

4. RESULTS AND DISCUSSION

4.1 Freshwater species

4.1.1 *Haplochromis* spp.

Being a small species it was expected that *Haplochromis* would spoil fast. In fact, after 7 hours of storage at ambient temperature fish smelled slightly acid and was definitely spoiled after 20 hours.

As far as spoilage of chilled *Haplochromis* is concerned, on the fifth day of storage iced *Haplochromis* developed a slight change of smell, and subsequently a progressive stronger stale-odour as well as ventral ruptures. Fish samples were rejected by the taste panel on the seventh day of storage mainly because of strong stale-odours, rancid taste and soft texture.

CLW samples kept slightly better than iced. They were rejected by the taste panel on the eighth day of storage, mainly because of the pronounced mushy texture and spoiled-odour.

Similar results were obtained by Meynell (4) in his experiments with *Haplochromis* spp. from Lake Malawi. However, they are not compatible with the unpublished results of Clucas and Pinegar (1973-74) that under ideal conditions *Haplochromis* spp. will remain acceptable between 15 and 21 days in ice. The possible reasons for the reported differences in the spoilage rate of iced *Haplochromis* might be due to differences in size and fat content.

Spoilage rate of *Haplochromis* during the experiment is shown in Figure 1.

Determination of TVB gave different results from those found in the very limited literature on the subject relating to tropical fish. The initial TVB value for fresh *Haplochromis* was 51.8 mg N-TVB/100 g, which is much higher than usually found in fresh fish. For example, Meynell (4) found only about 7 mg N-TVB/100 g in fresh *Haplochromis* spp. from Lake Malawi. The same range of TVB value has also been found for fresh tilapia and other freshwater tropical fishes.

Usually, during storage of fish, TVB value rises due to autolytic and bacterial spoilage. This was indeed observed during storage of *Haplochromis* at room temperature, when TVB value rose within one day from 51.8 mg N-TVB/100g to 91.4 mg N-TVB/100 g. However, in two other cases, when *Haplochromis* was stored in ice and chilled lake water, a different trend was observed. In both cases, TVB value, instead of increasing, had a clear tendency to decrease (Figure 2), dropping from 51.8 mg N-TVB/100 g at the beginning of the experiment to 16.3 mg N-TVB/100 g after ten days of storage in ice and 20.6 mg N-TVB/100 g after 16 days of storage in chilled lake water.

The explanation for these phenomena can be that volatile bases were leached out during storage. As a matter of fact, the melting rate of ice during the experiment was high and ice had to be replenished three times a day. However, a similar trend was observed also in the case of Nile perch fillets which were packed in plastic foil and the leaching effect was rather marginal.

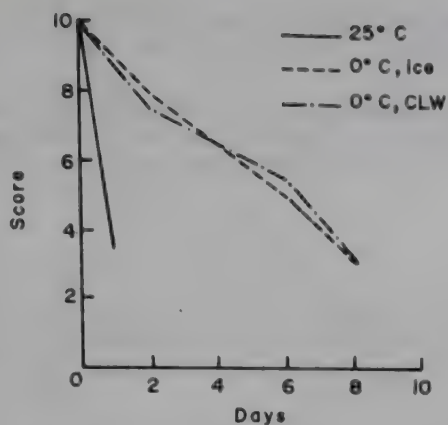


Figure 1 Spoilage rate of *Haplochromis*

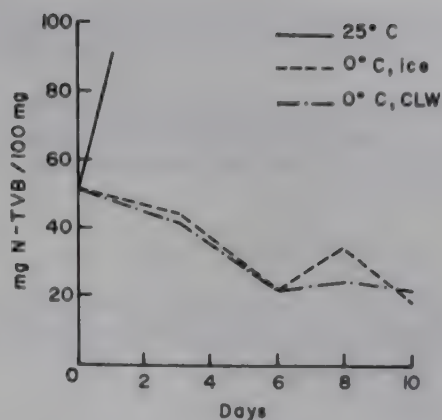


Figure 2 Changes in TVB value during storage of *Haplochromis*

4.1.2 Lungfish (*Protopterus ethiopicus*)

Lungfish, is of great interest to biologists because of its unique habit of breathing and behaviour in building nets and in hibernation. Its behaviour under fresh and iced storage conditions was previously studied by Hoffman *et al.*, (5).

During this experiment one third of lungfish kept at ambient temperature were still alive 24 hours after being caught. Samples, however, gave the impression of complete spoilage (also recorded by Hoffman *et al.*), due to the strong odour and large amount of surface slime, but once this was removed, the flesh was firm and had an acceptable odour until the third day of storage, when the samples were rejected by the taste panel. At that stage, a marked change of texture took place, the body of the fish distended, maggots appeared on the skin in contact with the box; there was a strong putrid odour and repugnant taste.

Lungfish put in ice or in CLW died quickly from temperature shock and their quality remained good until the seventh day of storage. Most of the panelists observed that flavour of even fresh lungfish meat differs from the other species, having a metallic slightly bitter after-taste, reminiscent of shark meat. After seven days, the iced samples started to develop a softer texture whereas the CLW samples kept a normal texture for a few more days. Iced samples were rejected by the taste panel on the 13th day of storage and the CLW samples on the next day (Fig. 3).

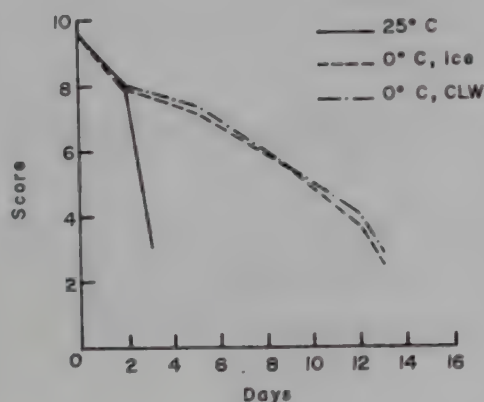


Figure 3 Spoilage rate of lungfish

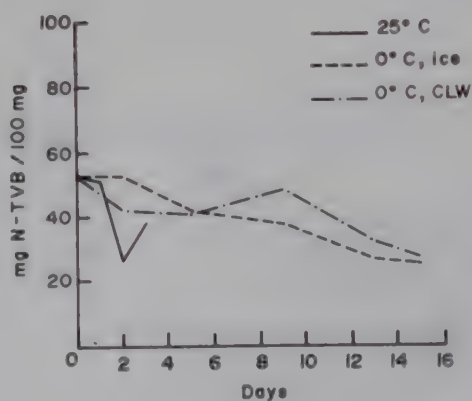


Figure 4 Changes in TVB value during storage of lungfish

During preparation of samples for organoleptic evaluation, the amount of cooking drip was visibly higher towards the end of the experiment.

The estimation of TVB in lungfish stored in different conditions gave similar results to those for *Haplochromis* spp. Again, the initial value was high (52.2 mg N-TVB/100 g) and the decrease during storage in ice and chilled lake water was observed (Fig. 4).

4.1.3 Nile perch (*Lates* spp.)

Nile perch, is a large predatory species. It may grow to over 120 kg. However, the specimens used in the present experiment were just 10-15 kg approximately. Nile perch caught in Lake Kyoga in Uganda is reported to keep in ice for 20 days, Hoffman *et al.*, (5).

On the second day of storage at room temperature, samples of Nile perch fillets were spoiled and were rejected by the taste panel. The fillets were dark in colour and all packs showed an excessive amount of drip.

Iced fillet packs showed the first signs of spoilage on the eighth day of storage when all samples gave signs of stale-odours and a softening in the texture. From that day on, all samples increasingly showed an excessive amount of drip and were rejected by the taste panel on the fifteenth day of storage, mainly due to flesh discoloration (browning), jelly-like or very soft texture and strong odours and bad taste (spoiled, rancid, stale). The same changes were observed for the CLW fillet packs but with less intensity, which made the taste panel reject them five days after, on the twentieth day of storage (Fig. 5).

Estimation of TVB in Nile perch fillets gave again high initial value of 48.5 mg N-TVB/100/g which in case of fillets stored for two days in ambient temperature rose up to 60.2 mg N-TVB/100 g. In case of samples stored under chilled conditions again decreasing trend was observed (Fig. 6), which this time cannot be explained by leaching effect, since the fillets were stored in tightly closed plastic bags. It seems that this problem requires further investigations.

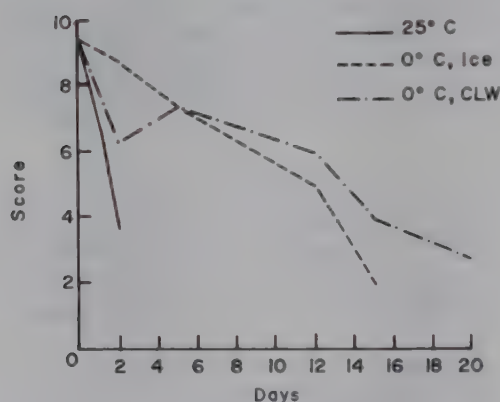


Figure 5 Spoilage rate of Nile perch fillets

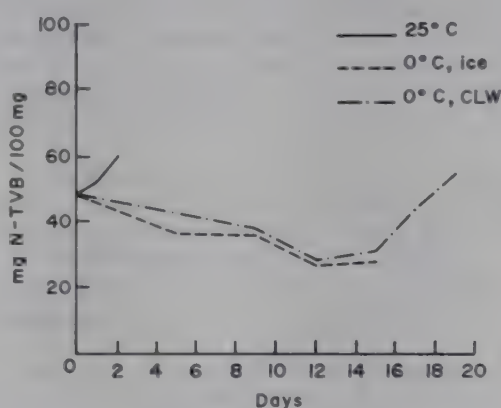


Figure 6 Changes in TVB value in Nile perch fillets

4.2 Marine Species

4.2.1 *Sardinella* (*Sardinella aurita*)

Sardines used in the experiment were lean, with a fat content of 1.25%.

Samples of fish left at ambient temperature were rejected on the second day of storage.

During the first week of storage there was no difference in quality between fish

kept in ice and chilled sea water (CSW). Later, the spoilage rate of fish kept in ice became faster and fish kept in ice became inedible after 14 days of storage.

Sardinella kept in CSW remained edible for 3 more days and was rejected on the 17th day of storage. From the 12th day onward increased salinity of meat was noted in fish stored in CSW. Changes in salt content are given in Figure 7 and spoilage rate in Figure 8.

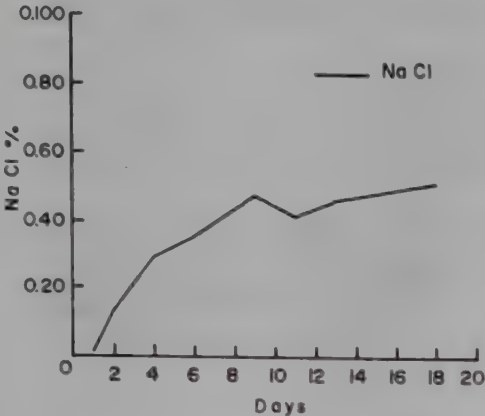


Figure 7 Changes in salt content of sardinella kept in CSW

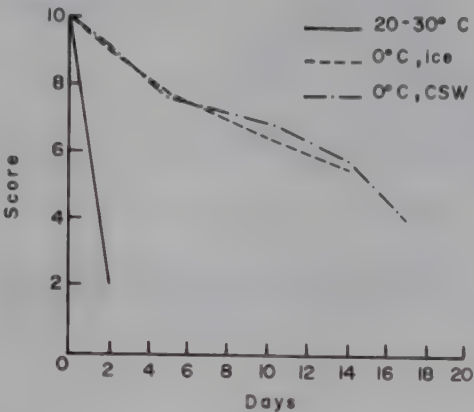


Figure 8 Spoilage rate of sardinella

As far as TVB are concerned, usual increasing trends have been observed correlating reasonably well with organoleptic evaluation (Fig. 9).

Total number of bacteria found on the skin of sardinella was high from the beginning. Though sample "0" was unfortunately lost the number of bacteria on the second day was within the range of 10^6 and 10^7 respectively for sardinella kept in CSW and ice. At the end of storage life (14 days in ice, 17 days in CSW) samples have reached the level of 10^{10} in 1 g.

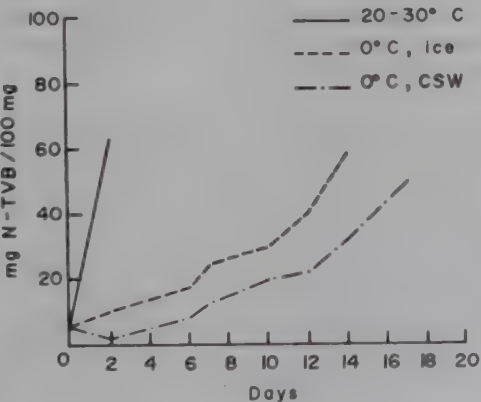


Figure 9 Changes in TVB value in sardinella

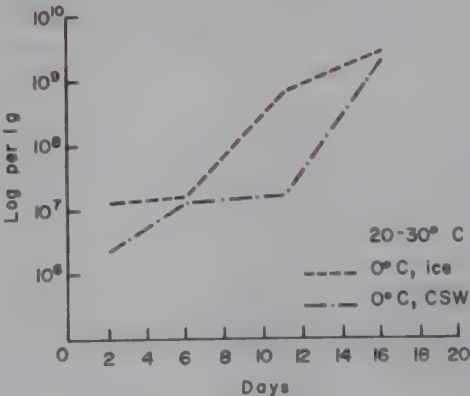


Figure 10 Changes in total bacteria count in sardinella during storage (per 1 g of skin)

4.2.2 Seabream (*Pagellus coupei*)

Similar to other species seabream kept at room temperature were spoiled on the day after catching. Samples stored in ice and chilled sea water had a storage life of 13 and 18 days respectively. Spoilage rate is given in Figure 11.

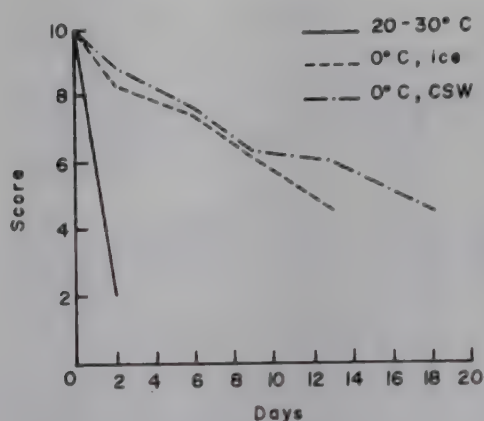


Figure 11 Storage life of seabream

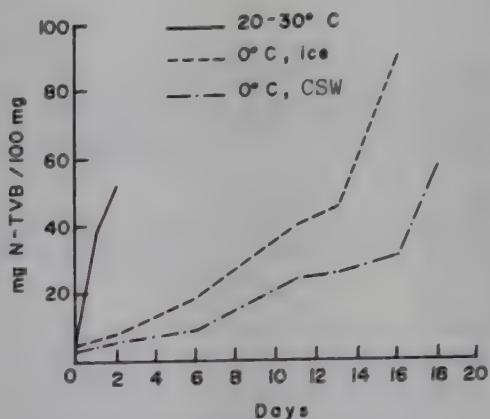


Figure 12 Changes in TVB value in seabream

Changes in TVB value in stored samples were very similar to those observed in the case of sardinella. Values reached at the point of rejection were 45.71 mg N-TVB/100g for 13 days of storage in ice and 57.85 mg N-TVB/100g for 18 days storage of sardinella in CSW.

Also the number of bacteria on the skin rose during storage from 10^7 and 10^6 per 1 g on the second day of storage up to 10^9 per 1 g and 10^{10} on 13 and 18 days of storage in ice and chilled water respectively.

5. CONCLUSIONS

Spoilage of tropical fish is fast at ambient temperature. This limits distribution of fresh fish to a narrow belt along the sea or lake coast. As has been shown in this experiment, the application of ice or chilled lake or sea water extends shelf-life of *Haplochromis* spp. up to eight days, lungfish up to 13 or 14 days, Nile perch fillets up to 14 and 19 days, sardinella up to 14 and 19 days and seabream 13 and 18 days respectively. No significant differences in storage life of freshwater species kept in ice or chilled lake water have been found. However, use of chilled seawater extended storage life of marine species up 5 days more than icing.

Use of chilled sea/lake water seems more advantageous, particularly if insulated containers are used. In the conditions of the experiment, to maintain the temperature of iced fish held in plastic fish boxes at 0°C, replenishment of ice at least three times a day was necessary. In the case of insulated containers, on the other hand, ice did not have to be added frequently but only every third day, still maintaining the temperature at 0°C. Use of these containers, therefore, would enable the transport of fish to be made much further inland without having to use refrigerated or insulated trucks. The consumption of ice in the case of containers is significantly lower than when fish boxes are used. Although the cost of 70-litre containers is about two and a half times higher than that of fish boxes of the same capacity, the lower ice consumption and better insulation properties make the use of containers more advantageous.

The amounts of TVB in freshwater species stored in ice or chilled lake water clearly showed a decreasing trend in all species investigated, even those (*Lates* spp.) which had no direct contact with the melting ice or chilled lake water. Probably the formation of TVB at temperatures around 0°C is significantly reduced or

halted, and spoilage takes a different pathway from what it does at higher temperatures. A similar effect of chilling on the formation of TVB in muscle of freshwater fishes has been observed by Moriyama (6) who found great differences in the TVB value among freshwater fish species in the early stage of spoilage. These, together with the results of this experiment, indicate that, further research on this matter is still required.

REFERENCES

1. F. BRAMSNAES (1965), Handling of fresh fish. In: Fish as food, Vol IV, pp. 5-63, Borgstrom, G (ed) New York: Academic Press, pp. 518
2. C.M.A. LIMA DOS SANTOS (1981), The storage of tropical fish in ice. Trop.Sci. 23 (2) pp. 97-127
3. C.M.A. LIMA DOS SANTOS, D. JAMES, F. TEUTCHER (1981), Guidelines for chilled fish storage experiments - FAO FIIU/T210
4. P.Y. MEYNELL (1979), Effect of pre-freezing handling procedures on the quality of Chisawasawa (*Lethrinops* and *Haplochromis* spp.) from Lake Malawi. Trop.Sci. 21 (2)
5. A. HOFFMAN, *et al.* (1974), The preservation of some East African freshwater fish. Afr.Y.Trop.Hydrobiol.Fish, 3(1):1-13
6. S. MORIYAMA (1964), Studies on putrefaction tests of fish and their application to freshwater fishes. Report 1. Determination of the volatile basic nitrogen. J.Naval Med.Assoc. 15(1):72-79

DUREE DE CONSERVATION PAR REFRIGERATION D'ESPECES AFRICAINES DE POISSON

RESUME : L'étude de la durée de conservation d'espèces de poisson tropicales maintenues à la température ambiante, aussi bien que dans de la glace ou dans de l'eau de lac ou de l'eau de mer glacée, indique que les espèces d'Haplochromis peuvent être maintenues à l'état réfrigéré à 0° C pendant 7-8 jours, le Protopterus ethiopicus pendant 13-14 jours et les filets de perche du Nil (Lates spp.) 15 à 20 jours. L'allache (Sardinella aurita) restait bonne pour la consommation pendant 14 jours lorsqu'elle était maintenue dans de la glace et 17 jours dans de l'eau de mer refroidie et le Pagellus coupei 13 et 18 jours, respectivement.

SOME OBSERVATIONS ON THE SPOILAGE OF TROPICALLY
CAUGHT FISH AT DIFFERENT STORAGE TEMPERATURES

W.F.A. HORNER

School of Food Studies, Humber College of Higher Education,
Grimsby, (United Kingdom)

J. CASTRO REAL:

D. REYNIER VALDEZ

E. ROCHA MILLER

Instituto Tecnológico del Mar, Boca del Rio, Veracruz, (Mexico)

INTRODUCTION

In spite of major improvements in the quality of Mexican marine fish landings due to the widespread use of ice to chill the catch on board, inefficient chilling and poor handling lead to poor quality and high wastage rates in some fish markets ashore.

It has been noticed that by the end of a fishing voyage in the tropics, bulk stowage in (originally) finely divided iced resembles a large-scale rigid "honeycomb" with each individual fish sitting in its own individual air pocket "dome". In consequence, a temperature gradient is set up across the fish, from 0°C at its points of contact with the ice below to the ambient temperature of the air within the pockets. The non-uniform application and intermittent use of ice between landing and retailing fish ashore cause further quality variability.

MATERIALS AND METHODS

Fish. Sierra or Spanish mackerel (*Scomberomorus maculatus*) and Mojarra (*Diapterus plumieri*) were selected on grounds of abundance and popularity, the former representing oily fleshed pelagic species, and the latter lean fleshed demersal species. Additionally, both species, could be obtained in fresh, pre-rigor condition direct from local line and beach-seine fishermen.

Storage conditions. Whole uneviscerated samples of each species were stored at 0, 8 and 22°C: in melting ice (after pre-cooling the fish to avoid "doming"), in water in a refrigerator (copying conditions found in common usage (Potey Leon, 1982/1/1) in markets for chilled storage) and in water ambient temperature (22°C ± 3°C Veracruz, October/November). The "ambient" samples for hypoxanthine determinations were held at 30°C ± 3°C (Veracruz, September) in damp cotton wool.

Sensory trials. The schemes shown in Tables Ia and Ib were developed after six months' usage of the scoring system described by Shewan et al. (1953)/2/ and used by a trained panel to assess sensory quality of duplicate samples at regular intervals over the storage periods. Cooked fillet samples were assessed after 20 minutes steaming.

Total viable count. Sections of muscle removed aseptically were homogenised in sterile peptone water. Serial dilutions were inoculated into plate count agar. Plate counts were made after incubating for 48 hours at 37°C.

TMA-N determination. An approximately 25 g. dorsal strip of muscle (avoiding the red muscle, in the case of sierra) homogenised 1:3 in 5% trichloroacetic acid was used for each TMA-N determination. The modification of Dyer's (1965)/3/ method proposed by Tozawa et al. (1971)/4/ was used.

Hypoxanthine determination. A similar strip of muscle chopped in a cooled container provided samples for homogenization with ice-cold 5% perchloric acid which were filtered and neutralized according to the procedure of Jones and Murray (1962)/5/ and the hypoxanthine concentrations determined using the manual enzymic method of Jones et al. (1964)/6/.

Table 1a Sensory Assessment Scheme: Hojarra plateada (Diapterus plumieri)

SCORE CHARACTERISTIC	CLASS I			CLASS II		CLASS III		CLASS IV			
	10	9	8	7	6	5	4	3	2	1	0
APPEARANCE	The same as in life black pupils and transparent cornea			Slight opalescence in the cornea and pupil becoming grey		Cornea translucent rather than transparent Pupils grey and loss of turgor causing sunken-eyes		Eyes flacid and sunken Redness in the cornea and bacterial slime cover			
SCALES AND SKIN	Scales adhere strongly to the skin which is shiny & opalescent			Scales not easily removed from skin which has lost its initial brilliance		Scales easily removed and bacterial slime becoming apparent. Skin becoming dull		Scales fall off easily and the skin can be pulled off easily. Excessive bacterial slime.			
GILLS	Brilliant blood red living colour			Reddish brown no bacterial slime		More brown or even greyish with some bacterial slime		Greyish bleached pink colour with bacterial slime more obvious			
ODOUR	Sweet but faint odour of seaweed or shrimps			Natural or still slightly sweet		Yeasty, mousey, acetaldehyde		H ₂ S, Indole, ammonia lactic acid-smell drains or sewers			
FLESH	Firm and elastic to finger touch			Firm but with some loss of elasticity		Soft enough to leave marks when pressed with the finger		Very soft, almost like soap which has been left in water			
RAW INTEGRITY	Blue white translucent meat which holds together well, is firm and elastic			Firm but with a more wax-like appearance than translucence		Loss of blue whiteness replaced by wax-like appearance		Opaque, wax-like and falls apart easily			
COOKED FILLET	Firm flesh which remains in one piece and has a sweet shrimp-like taste			Firm but a little dry and more ready to fall apart when touched. Flavour bland slightly sweet		Open texture which breaks easily. Flavour lacking - like wool or cotton		Fillets break up during cooking and unpleasant flavours: bitterness, sourness and putrescence are apparent			



Sierra in a fragment of crushed block ice stowage showing typical doming effect

Table 1b Sensory Assessment Scheme: Sierra (Scomberomorus maculatus)

SCORE CHARACTERISTIC	CLASS I			CLASS II		CLASS III		CLASS IV			
	10	9	8	7	6	5	4	3	2	1	0
APPEARANCE	Bright and clear with black pupils as in living fish			Some loss of brightness and slight greying of the pupil but no loss of turgour		Pupil becoming grey and cornea becoming opaque. Definite loss of turgour		Grey pupil, opaque cornea Eyes sunken and covered with bacterial slime			
SKIN AND FINS	Shiny living appearance with opalescent sheen and clear bright-yellow markings. Anterior dorsal fin complete and firm			Some loss of opalescence and brightness of the yellow markings. Anterior dorsal fin complete but easily broken		Yellow markings lack lustre and some evidence of slime formation along the lateral line Anterior dorsal fin breaks on the finger		Bacterial slime covers the surfaces and the yellow markings are no longer clear. The anterior dorsal fin falls apart when lifted			
GILLS	Living blood red to flesh pink colour			Red but dull, lack-lustre		Red-brown with some evidence of slime		Brown or greyish and covered with bacterial slime			
ODOUR	Neutral or seaweed-like or sharp seawater odour			Neutral - no particular marine associated odour		Slight fish oil odour but not disagreeable		Strong rancid, lactic acid or proteolytic odours. Possible presence of lower nitrogen compounds			
FLESH	Firm and elastic does not retain finger imprints			Firm but some loss of elasticity, retains finger imprints		Becoming soft and without elasticity. Loss of gut integrity		Flesh breaks up easily and loses oil when pressed			
RAW FILLET INTEGRITY	Blue-white translucent and firm flesh with a red mark along the length of the spine			Little loss of blue translucence but growth of red area close to the spine		Opaque white and soft meat which breaks easily and is discoloured where the fillets touched the viscera		Opaque almost yellow with marked discolouration along where the fillet touched spine or viscera			
COOKED FILLET	Firm meat with a sweet tuna or chicken-like flavour; very delicate			Firm meat which tends to fall apart when handled. Meaty flavour		Meat a little dry due to loss of oil. Slight bitterness detectable		Fillets break up when cooked. Sour, bitter or even putrid flavours predominate			

Figure 1

Sierra

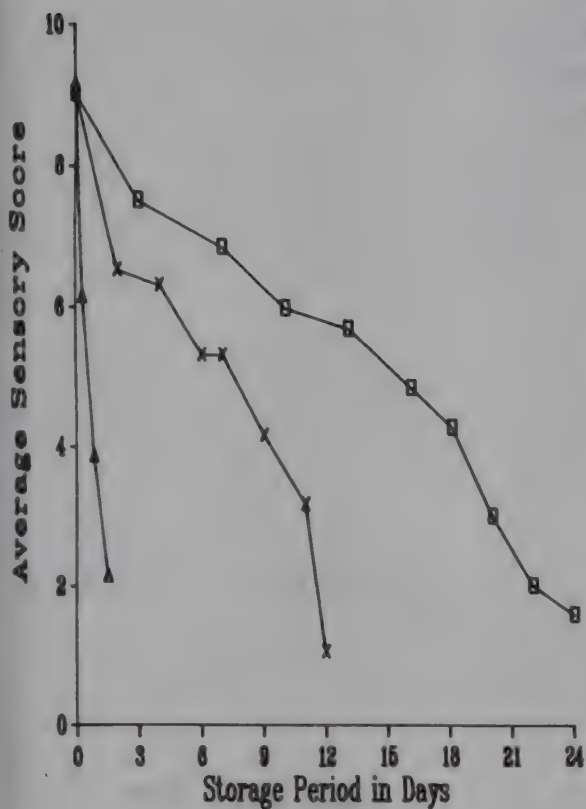


Figure 2

Mojarra

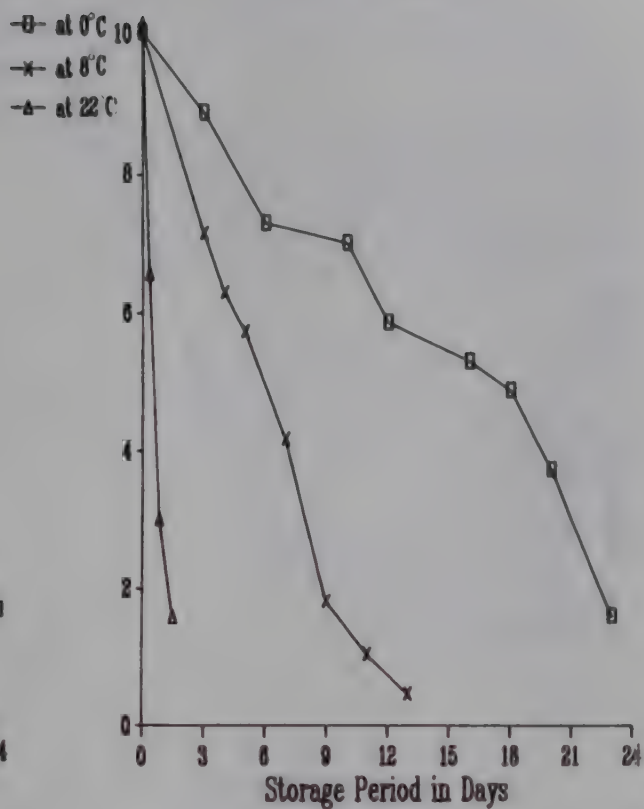


Figure 3

Sierra

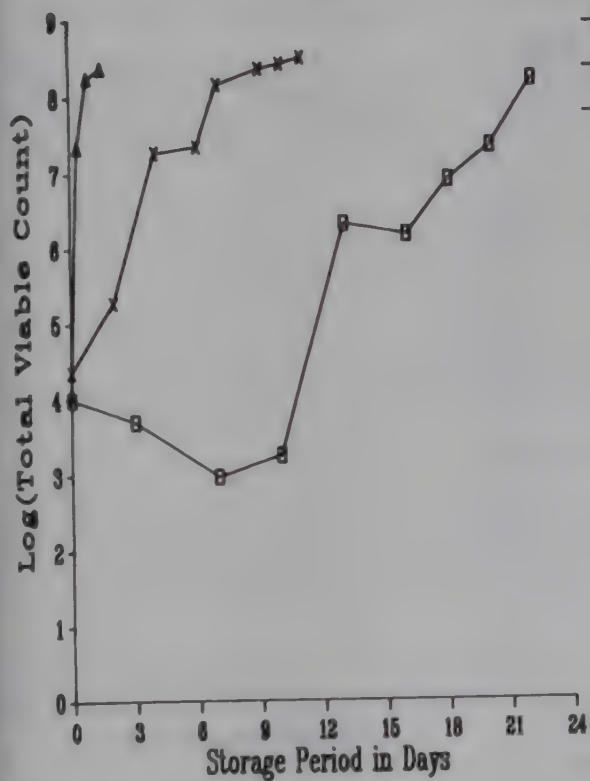


Figure 4

Mojarra

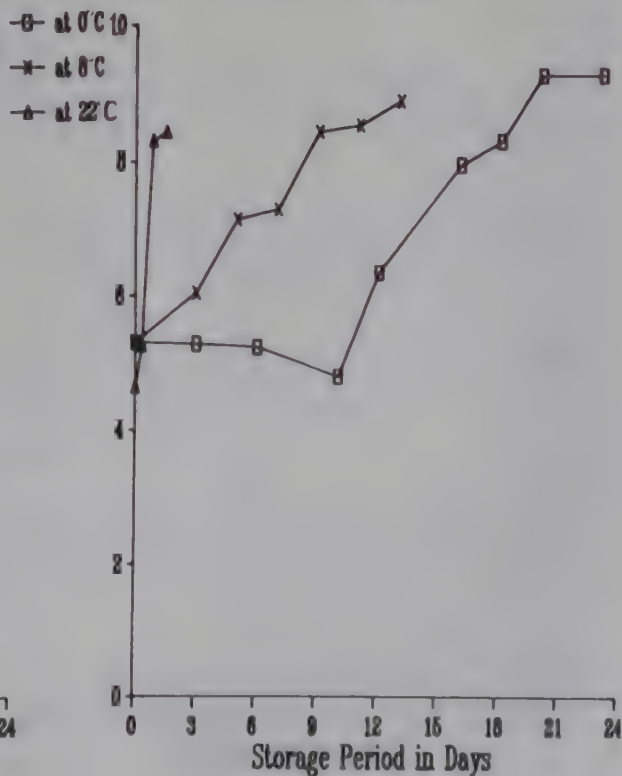


Figure 5

Sierra

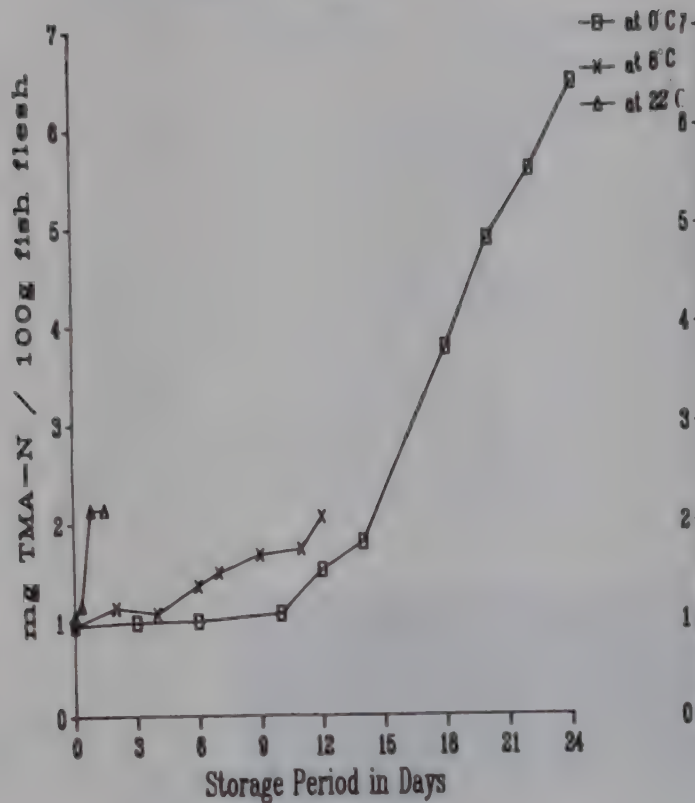


Figure 6

Mojarra

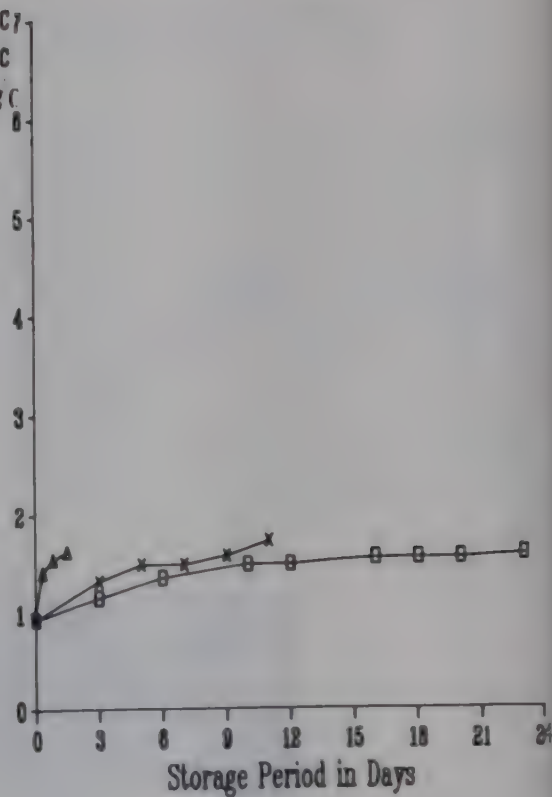


Figure 7

Sierra

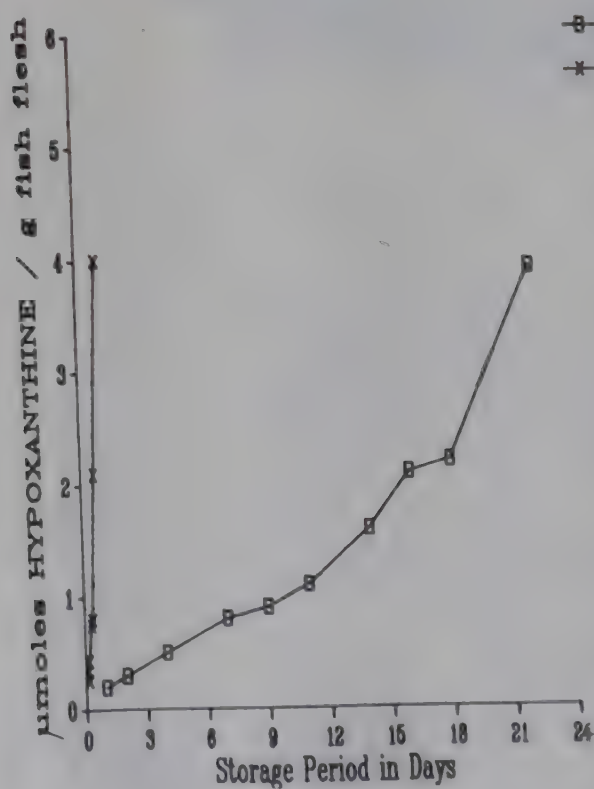
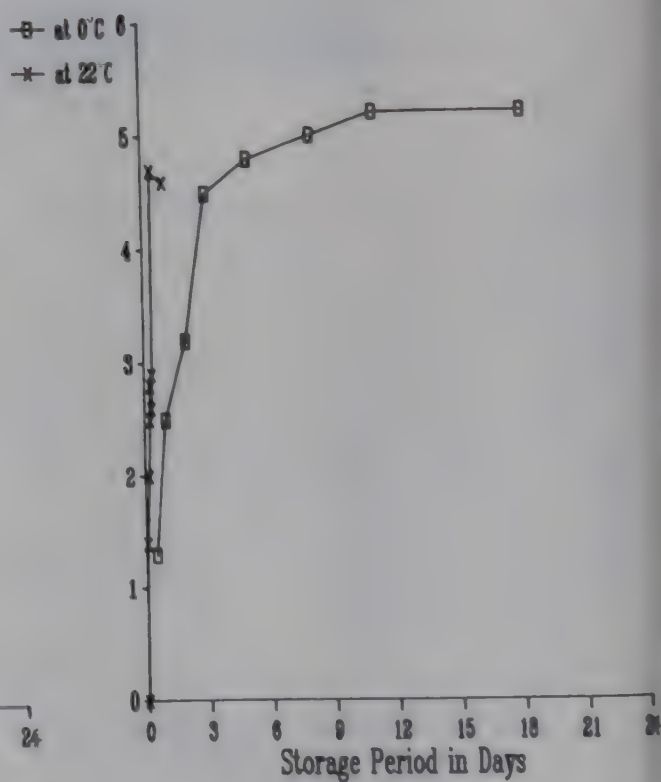


Figure 8

Mojarra



RESULTS AND DISCUSSION

Sensory assessment. Individual preferences may not necessarily concur with the assigned grades I, II, III, IV approximately representing EEC grades E, A, B, C. For example "meaty" or "savoury" flavours of Grade III samples might, to some, be desirable. Accordingly, the minimum fish quality score regarded as acceptable for human consumption might be taken as 6 or 4.

Figures 1 and 2 show the change in average sensory score (the total sample cumulative score divided by the number of panellists, divided by 7, which was the number of sensory attributes examined) with time of storage at the different temperatures used. Correlation coefficients for the graphs fall almost entirely between - 0.95 and - 0.98 indicating the same approximate degree of reliability in storage life prediction as Connell and Shewan (1979) 7/ claim for the Torry scheme.

Individual Spoilage Rates (I.S.R.) taken as the gradients of the graphs for cooked fillet flavour score against time, were 0.30 and 0.52 for sierra at 0 and 8°C respectively, and 0.31 and 0.78 for mojarra at 0 and 8°C. Over the temperature range 0 to 8°C the I.S.R. might be expected to increase by a factor of 3.2 (using $z = 25^\circ\text{C}$). The obtained factors of 1.73 and 2.52 for sierra and mojarra respectively indicate the dubious validity of using a scheme developed for 0°C spoilage to describe spoilage at higher temperatures. Such discrepancies were even more noticeable for the 22°C stored samples. For example, panel average appearance scores for these samples were often 2 points and, sometimes, 3 points higher than odour, texture and taste scores. This was due to the early detection of acid notes, supporting similar findings for mesophilic spoilage reported by Gorczyca et al. (1985)/8/. A consequence of this score discrepancy is that fish kept at tropical ambient might be passed as acceptable on its appearance when unfit on taste attributes.

Total Viable Counts. The initial decline in TVC shown by the 0°C stored samples (Figures 3 and 4) coincided with a change in microbial flora from mixed Gram-positive and Gram-negative cocci and bacilli immediately after capture to, predominantly Gram-negative bacilli at the end of 4 days of iced storage. This, together with the subsequent long lag period, might contribute to the relatively greater extension of shelf-life produced when tropical species are stored in ice compared to temperate species noted by workers in tropical fisheries (Jones and Disney (1977)/9/. The results, however, indicate quality predictive usefulness for the higher storage temperatures.

TMA-N. Connell and Shewan (1979)/7/ state that all methods for freshness assessment based on the products of microbial activities are only applicable in the stages of spoilage where bacterial numbers rise sharply. The results summarised by Figures 5 and 6 bear out this statement.

Leaching, particularly from the non-scaly sierra samples stored in water at 8°C and 22°C, appears to have occurred. The ambient-stored samples for hypoxanthine determination were therefore protected from dessication using damp cotton-wool.

Hypoxanthine. From the results summarised in Figures 7 and 8 it would seem that hypoxanthine concentration might provide a satisfactory index of the spoilage of sierra. In mojarra, however, the hypoxanthine concentration might provide a reliable quality index only over the first 3 to 4 days of iced storage.

CONCLUSION

Chemical tests could provide useful objective supports to sensory testing as part of commercial quality assurance procedures for fresh fish in the tropics. A Q.A. programme would need to make allowances, however, for sources of variation additional to those encountered in a similar temperate operation due to:

- i) temperate gradients in individual fish as a result of the "doming" phenomenon in bulk stowage;
- ii) leaching of spoilage indicator substances into the melt-water at the bottom of non-draining ice boxes;
- iii) different spoilage rates in flesh adjacent to and distant from the viscera of whole fish.

REFERENCES

1. J.L. POTEY-LEON: Unpublished student thesis, Instituto Tecnológico del Mar, Boca del Rio, Veracruz, Mexico. (1982).
2. J. M. SHEWAN, RUTH G. MACKINTOSH, C. G. TUCKER & A. S. C. EHRENBURG:
The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. *J. Sci. Fd. Agric.* Vol. 4 (1953) pp. 283-289.
3. W. J. DYER: Amines in fish muscle. I Colourimetric determination of trimethylamine as the picrate salt.
J. Fish Res. Bd. Can. Vol. 6 (1945) pp. 351-358.
4. H. TOZAWA, K. ENOKIMARA & K. AMANO: Proposed modification of Dyer's Method for trimethylamine determination in cod fish. *Fish inspection and Quality Control.* E. R. Kreuzer. FAO Fishing News Books Ltd. (1971) pp. 187-190.
5. N. R. JONES & J. MURRAY: Degradation of adenine and hypoxanthine nucleotide in the muscle of chill-stored trawled cod (*Gadus callarius*)
J. Sci. Fd. Agric. Vol 13 (1962) pp. 475-480.
6. N. R. JONES, J. MURRAY & (in part) E. I. LIVINGSTON & C. K. MURRAY:
Rapid estimations of hypoxanthine concentrations as indices of freshness of chill-stored fish. *J. Sci. Fd. Agric.* Vol. 15 (1964) pp. 763-774.
7. J. J. CONNELL & J. M. SHEWAN: Past, present and future of fish science. *Advances in fish Science and Technology.* Ed. J. J. Connell. Fishing News Books Ltd. (1980) pp. 56-65.
8. E. GORCZYCA, J. L. SUMNER, D. COHEN & P. BRADY: Mesophilic Fish Spoilage.
Fd. Technol. Australia Vol. 37 (1) 1985 pp. 24-26.
9. N. R. JONES & J. G. DISNEY: Technology in fisheries development in the tropics. *Proceedings of the conference on the handling, processing and marketing of tropical fish.* Tropical Products Institute (1976) pp. 27-31.

QUELQUES OBSERVATIONS SUR LA DETERIORATION DES POISSONS TROPICAUX.

RESUME : Quand on place des poissons d'eaux tropicales (25 - 30°C) dans la glace finement divisée, la fonte rapide mène à la formation d'un dôme de glace de telle sorte que chaque poisson se trouve dans une poche d'air avec seule sa partie inférieure en contact avec la glace. Les baisses de température ambiante qui se produisent en conséquence, affectent le taux et la forme de détérioration.

Cependant, il convient d'admettre certaines tolérances en raison de sources de variations plus importantes que celles rencontrées dans des conditions similaires en milieu tempéré.

DISCUSSION

R.G. POULTER (U.K.) - I have some observations which the author of the present paper and Dr Karnicki who presented the previous paper may wish to comment on. There is now a fair amount of evidence to suggest that tropical fish generally are more stable to post capture abuse than cold water fish. Peter Howgate has just mentioned the fact that some tropical fish can be stored at tropical ambient temperatures for many hours and still have an acceptable shelf-life in ice. Similarly there is evidence that many species of fish found in the tropics will remain acceptable in ice for extended periods. For example, in a recent visit to Latin America I was shown the results of a study that suggested some of the fish caught in the Amazon area may remain acceptable to the consumer for over 40 days. Evidence is also building up to indicate that some tropical fish species are particularly stable during freezing and frozen storage.

The point which I would like the authors to comment on is that in handling and processing fish from tropical waters it is important to adopt techniques which are designed to give optimum quality and yields for those particular species. At present the codes of practice for fish processing are largely based on experience with cold water species (particularly gadoids); these may need to be modified if they are to be applicable to tropical species.

W.F.A. HORNER - We did not conduct any studies involving keeping tropical fish at ambient and later storing in ice. The two particular species investigated did not appear to exhibit a storage life significantly longer than similar temperate water species. However, we are aware of studies, both in the literature and in final year degree projects at the Veracruz College, on chilled storage of tropical species where very long storage lives have been claimed.

To achieve efficient cooling of tropical fish on-board, we believe that it may be necessary to take steps to counter ice-dome formation in the stowage. Perhaps in this respect tube-ice may be more efficient than flake or crushed block ice, for example.

M. JUL (Denmark) - The last two papers have related to fisheries development in the Third World and to the use of chilling and freezing, therefore, I hope it is realized that these are rather expensive preservation methods. If one distributes chilled or frozen fish, the cost of these means of preservation may easily exceed the food value of the fishery product itself, thereby it may bring the latter out of reach for the lowest income groups ie those normally considered the potentially malnourished and deprived.

In my view this does not mean that chilling or freezing are not suited for the Third World, only that their role should be properly understood. They may often be used as employment and income generating tools. They may result in exports, thereby bringing home badly needed foreign exchange, or they may create a sale to higher income groups, often primarily in cities. In so doing these technologies may create incomes for some very poor fishermen, workers in processing plant, supply firms etc. The contribution to development is not the fish that is preserved but the income that is generated.

W.F.A. HORNER - I agree wholeheartedly. However, additionally, in Mexico, there is a need to encourage more families who buy meat as the principle component of the meal to substitute this with fish occasionally. The Mexican Government have subsidised chilling and freezing of cheaper, less popular species, so that lower income groups may be encouraged to buy as well as higher income groups.

SHELF LIFE OF CHILLED ORANGE ROUGHY -
RESEARCH STRATEGY FOR A NEW FISHERY

G.C. FLETCHER, D.N. SCOTT, R.J. SEELYE
Division of Horticulture and Processing,
Department of Scientific and Industrial Research (New Zealand)

Orange roughy (*Hoplostethus atlanticus*) is a recent arrival on the New Zealand fishing scene with commercial quantities first landed in 1979 /1/. Since then the catch has increased dramatically to a 33,000 tonne quota in 1983. With exports at a value of 47 million dollars, orange roughy is currently New Zealand's most valuable finfish species /2/.

Presently the catch is landed in one of two forms, either whole on ice, or frozen, headed and gutted fish. The whole fresh fish are usually headed, filleted, skinned and sold either fresh or as frozen shatterpack fillets. The frozen fish are generally thawed, filleted, skinned, then refrozen as shatterpacks and exported. These shatterpacks are often thawed and consumer packaged before being retailed as chilled fillets. Unlike many fillets, those of orange roughy do not change in appearance when spoiled.

This paper presents an overview of work carried out since 1981 on the storage and shelf life of orange roughy. The research strategy described might be applied to another newly developed fishery where little or no information is available on optimum handling and shelf life.

1. RESEARCH STRATEGY

Many of the needs for research on orange roughy were unique. This was the first commercially successful deep water species to be exploited. For the first time New Zealand manned trawlers were fishing at 1,000 metres and very large catches were being achieved. To the processor, the frozen fish appeared to be very freeze-thaw stable and to have an indefinite shelf life. The retailer reported problems in determining the quality of the chilled fish and when to withdraw it from sale. A research strategy was clearly needed which could answer these questions in a methodical way. The first step was to establish some baseline data on the spoilage and shelf life of orange roughy. The aim was to determine the shelf life of the fish and to identify objective tests which correlated with results of sensory analysis.

Orange roughy of good appearance were selected from a trawl net and immediately iced. Initial samples were taken and the fish were transported to the laboratory where 5 fish were removed at regular intervals for sensory, chemical and microbiological analyses. The colour, surface slime, appearance of gills, odour of gills and texture of raw flesh were recorded. To determine the shelf life, samples of the orange roughy were cooked and their attributes were evaluated by the members of the taste panel described by Scott and Hogg /3/. Panelists described the odour, texture and flavour and rated the acceptability of each attribute.

Surface pH was measured using a surface pH electrode placed directly on the skeleton side of the fillet. Trimethylamine (TMA) was determined by the modified Dyer picrate method /4/. Adenosine triphosphate (ATP) and its degradation products were determined by HPLC /5/ and the K value /6/ was calculated. The absolute concentrations of nucleotides, nucleosides and bases varied significantly between different batches of orange roughy. Thus, it was necessary to determine the relative concentrations of these compounds to the total pool of purine derivatives in order to make comparisons between trials. Total aerobic plate counts (APC) were monitored in the anterior dorsal on the skin and flesh by pour plates of "Medium B" of Simidu and Hasuo /7/. A black colony agar was used to determine sulphide producing bacteria /8/. Previous work had shown that sulphide producing bacteria could become the dominant flora during storage of fish /9/.

The second step in the research strategy was to examine the effect of poor handling at sea on the shelf life. Large commercial catches of orange roughy had resulted in fish being landed on deck after severe crushing in the net and with subsequent delays before stowage in ice.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

The effect of heading and gutting at sea was also examined. Some workers have reported an extension of shelf life for other species as a result of gutting at sea prior to ice storage /10/, /11/. Since approximately one third of the fish by volume is head, heading and gutting would also allow more fish to be stowed. However this had to be offset against the value of the head as a source of a potentially high value wax ester /12/. Heading and gutting is carried out in New Zealand waters on many of the fish species that are frozen at sea.

The effect of filleting on the shelf-life was studied next. Filleting is the major cause of bacterial contamination of fish flesh /13/. The shelf life of fillets was unknown and retailers had reported difficulties in determining the quality by the appearance. Also, it was not known what effect storage in ice prior to filleting and freezing had on the subsequent quality of the fillets. Other workers have recommended delayed filleting of cod over holding fillets for the extra time /14/.

Most orange roughy is exported as frozen shatter-packed fillets. Thus, fish which are landed in frozen headed and gutted form are subsequently thawed, processed and refrozen. The effect of this second freezing was not known. A study was undertaken to compare the shelf life of fillets taken directly from frozen headed and gutted fish with fillets that had been refrozen.

Finally the shelf life of fillets at the retail end of the cold chain was examined. A temperature of 0°C is recommended for holding fish. Frequently however, the temperature may be 4°C or even as high as 9°C. The effect of these storage temperatures needed to be determined in order to provide data for written guidelines.

2. RESULTS AND DISCUSSION

Storage of whole fish in ice

The flavour of the cooked fish was the most important attribute determining the acceptability. Flavour profiles of fish stored in ice and a frozen reference are shown in Table I.

TABLE I
Flavour of cooked orange roughy stored in ice

DAYS OF STORAGE	4			6			9			11			13		
FLAVOUR TERMS	R**	W	HG	R	W	HG	R	W	HG	R	W	HG	R	W	HG
Sweet	1.00*	0.67	0.60	0.76	0.76	0.59	0.79	0.50	0.50	0.68	0.58	0.47	0.88	0.18	0.53
Salty	0.27	0.20	0.20	0.41	0.35	0.18	0.36	0.29		0.32	0.32		0.24	0.18	0.24
Sour			0.27	0.18				0.29	0.21		0.26			0.24	0.29
Bitter		0.20	0.20		0.18	0.29		0.29	0.21	0.21		0.21		0.41	0.24
Lamby/Meaty	0.27	0.20	0.27	0.29	0.29		0.43		0.29		0.21	0.26	0.41		0.24
Chicken						0.29	0.36		0.21	0.21	0.21		0.24		0.24
Shellfish				0.18			0.21								
Milky/Creamy	0.67	0.67	0.67	0.65	0.53	0.35	0.64	0.36	0.57	0.42	0.47	0.37	0.53	0.41	0.47
Buttery	0.20			0.24	0.18		0.29		0.21	0.26		0.26	0.24		0.24
Caramel	0.20														
Nutty	0.27			0.29	0.24	0.24			0.29	0.32			0.24		
Neutral/Bland	0.27	0.47	0.33		0.41	0.29		0.36	0.21	0.21	0.32	0.26		0.24	0.24
Mushroom								0.21						0.24	0.24
Cardboard														0.18	
Potato									0.21						
Musty														0.24	
Flounder										0.21					
Stale														0.29	
Metallic							0.36							0.18	

* Figures are the proportion of the 12-20 panelists using a flavor term. Proportions were recorded if greater than 0.175.

** R - Reference
W - Whole
HG - Headed and Gutted

The characteristic flavour of fresh orange roughy after cooking was milky/creamy, sweet, lamby/meaty and salty. Sweetness decreased during storage and sourness developed. After 13 days low acceptability scores were recorded and the flavour was described as bitter, sour, mushroom, cardboard, musty, stale and metallic. The shelf life was defined as the period prior to the development of flavour aspects which were disliked by the panelists. Thus, the shelf life of whole orange roughy stored in ice was 11 to 12 days.

The mean surface pH of the fish did not change significantly during the shelf life and significant increases in TMA did not occur until after the end of the shelf life. However, the inosine monophosphate (IMP) level did change, with a corresponding increase in the level of inosine (Fig. 1). Only small amounts of hypoxanthine were formed. The K value showed an approximately linear increase up to 56% at 11 days (Fig. 2).

Microbiological analyses showed a log linear increase in APC during storage reaching about 10^7 on the surface and 10^4 to 10^5 in the flesh at the end of shelf-life (Fig. 3). Sulphide producers were a very small proportion of the aerobes, reaching 10 in the flesh at the end of shelf life.

The appearance and odour of the whole fish changed markedly during storage (Table II). By considering a combination of the raw attributes it would be possible to estimate the quality of whole orange roughy as an equivalent days in ice.

TABLE II

Significant changes apparent in whole orange roughy stored in ice

Attribute	Change observed and period of change
surface colour	red/orange — 6-11 days —> bluish grey
surface slime	clear or slightly cloudy — 11-13 days —> brown — 13-16 days —> thick yellow
gill appearance	dark red — 6-11 days —> brown/red with slime
gill odour	slight seaweed — 4-9 days —> sweet salty — 11-13 days —> stale seaweed — 13-16 days —> putrid
flesh texture	firm and resilient — 9-11 days —> soft, retaining indentations

Fig. 1. Purine derivatives in whole orange roughy

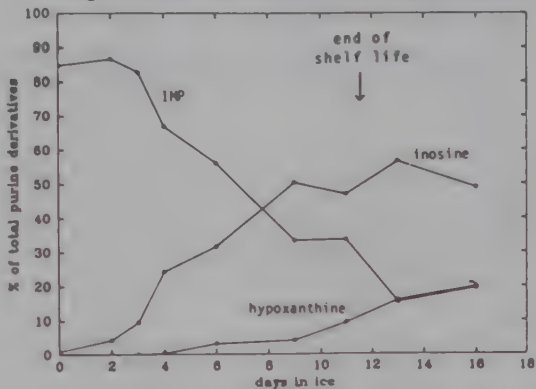


Fig. 2. K value of whole orange roughy

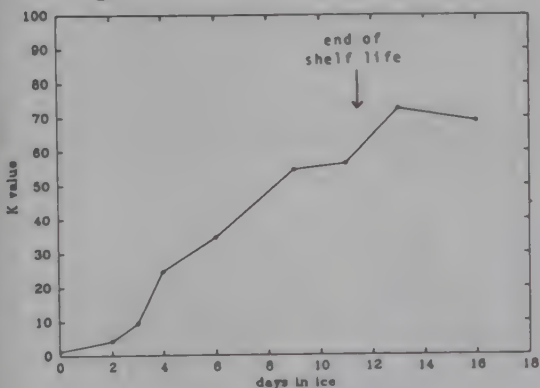
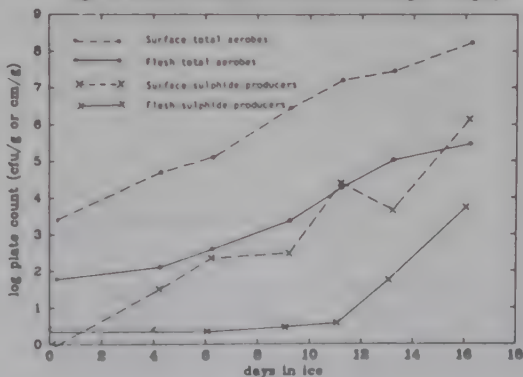


Fig. 3. Plate counts of whole orange roughy



Handling at sea

Trawl-damaged orange roughy stored in ice were found to have a shelf life of 9 to 10 days, or 2 days less than well handled orange roughy.

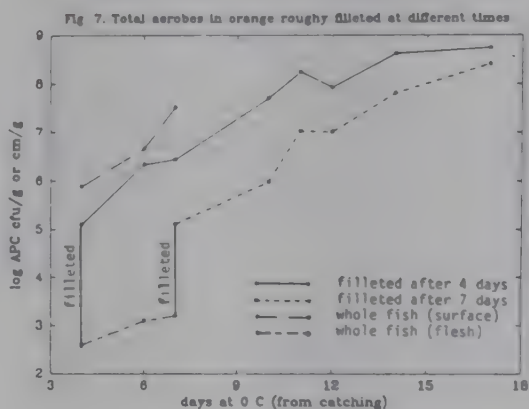
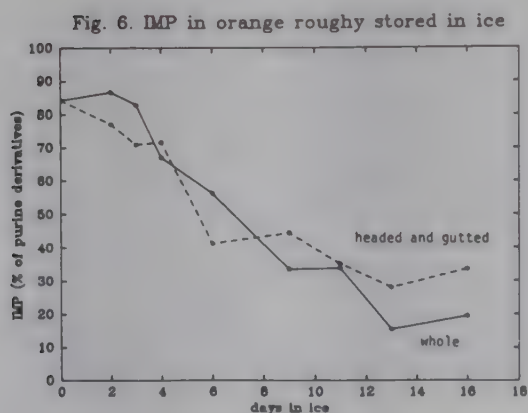
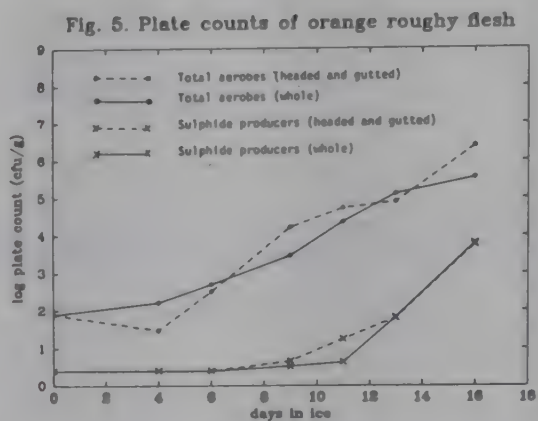
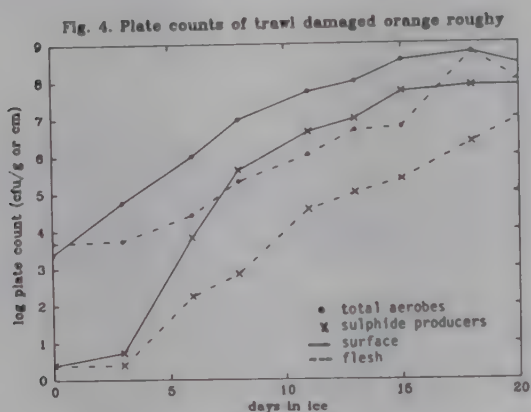
Microbiological results suggested that there had been a more rapid invasion of the flesh by bacteria as a result of the trawl damage (Fig. 4) compared to well handled fish. This was possibly due to loss of textural integrity in these fish. The proportion of bacteria in the flesh to that on the surface might be used as an indicator of handling.

Fig. 7 shows bacterial counts of fish caught, iced and landed under commercial conditions during a period of high catch rates when delays occurred prior to stowage in ice. The proportion of bacteria in the flesh compared to the surface suggests the fish did not undergo significant trawl damage. However, 4 days after catching they were judged by sensory evaluation to be equivalent to fish stored 10 days in ice. Surface APC's were higher in the commercially caught fish compared to the baseline data on orange roughy and there was a higher proportion of sulphide producing bacteria.

Heading and gutting

Heading and gutting orange roughy resulted in an extension of shelf life in ice of 2-3 days. Although no unpleasant flavours were reported after 13 days, by 16 days the fish were putrid and were not served to the panel (Table I). There were no consistent differences in bacterial counts of whole compared with headed and gutted fish (Fig. 5). Levels of IMP, inosine and the K value were significantly different at the end of shelf-life (Fig. 6). This suggests that the increased shelf life was a result of less autolytic spoilage, possibly due to the removal of enzymes in the gut region.

Commercial considerations suggest that this increase in shelf life is not sufficient to encourage heading and gutting at sea, which would involve extra labour, longer delays before chilling, and the loss of income from the fish heads.



The effect of filleting

Whole fish stored in ice were filleted under hygienic conditions after either 4 days or 7 days from catching. Fillets were held in polythene bags in ice. The development of off-flavours was used to determine the shelf life and this was an additional 7 days and 6 days for the fish filleted after 4 days and 7 days respectively. Beyond this time muddy, stale and musty flavours were reported. The appearance of the fillets did not change during storage but sensory evaluation showed that unpleasant odours developed in the raw fillets. These odours could be used as

a method to recognise the end of shelf life.

Bacterial growth from the day of filleting was similar for the 2 treatments with total aerobic counts exceeding 10^7 /g 5 days after filleting (Fig. 7). This is in agreement with the results of Shaw et al. /14/ who concluded that maximum overall shelf life could be obtained by delaying filleting. In our study, delaying filleting by 3 days resulted in an extension of the overall shelf life of 2 days. Autolytic spoilage was not affected by filleting and levels of IMP showed that autolysis was having some effect on the shelf life (Fig. 8).

The effect of freezing and thawing

Double freezing of orange roughy during normal commercial handling and processing caused a loss of 2 to 3 days in the shelf life compared to fish which had been frozen once. Bacterial results reflected this with a lag phase in the thawed fillets from frozen at sea fish (Fig. 9).

Fig. 8. IMP in orange roughy filleted at different times

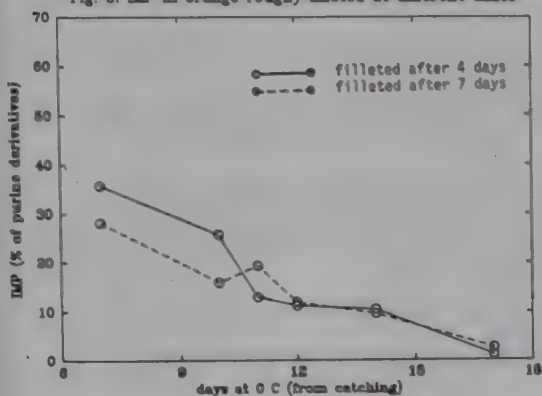
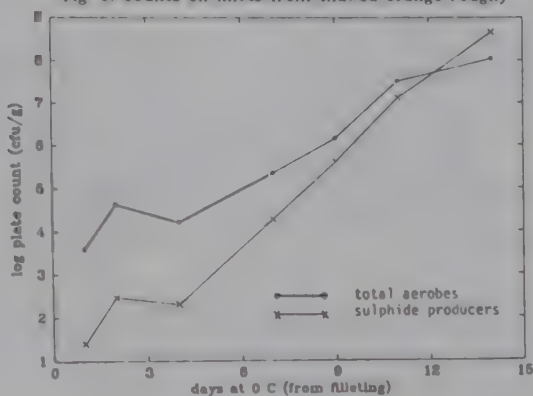


Fig. 9. Counts on fillets from thawed orange roughy



Effect of storage temperature

The shelf-lives of thawed fillets held at 4, and 9°C were 3 and 1-2 days respectively. The shelf life results were consistent with that obtained using a derivation of the Ratkowsky equation /15/ using T_0 of -10°C;

$$\text{shelf life at } T^\circ\text{C} = \frac{\text{shelf life at } 0^\circ\text{C}}{(0.1T + 1)^2} \quad \dots(1) \quad /16/.$$

Given the 3 day shelf life at 4°C the formula would predict shelf lives of 5.9 and 1.6 days at 0°C and 9°C respectively.

3. CONCLUSIONS

The research strategy used for this new species was to examine and compare different techniques of handling and processing used by industry. These results could be used to formulate guidelines and codes of practice to optimise the quality of orange roughy received by the consumer.

This strategy has provided detailed information about orange roughy at all stages of handling. Initially the storage of whole orange roughy in ice was studied. Procedures for monitoring changes in the fish were developed and subsequently applied in a series of studies on handling at sea, heading and gutting, filleting, freezing and storage.

With this base of information future problems concerning the handling and shelf life of orange roughy may be quickly solved.

4. REFERENCES

1. ROBERTSON, D.A.; GRIMES, P.J.: The New Zealand orange roughy fishery. Proc. FRD/MAF conference "New Zealand finfish fisheries" (1983). Trade Publications Ltd, Auckland.
2. NEW ZEALAND FISHING INDUSTRY BOARD: Fish export statistics, Jan-Dec 1983, world analysis by country and species (1984). NZFIB Wellington.
3. SCOTT, D.N.; HOGG, M.G.: Development and use of a taste panel for the determination of shelf life. These proceedings (1985).
4. ANON: Recommended general methods for the examination of fish and fish products. Analyst (1979) 104: 444.
5. RYDER, J.M. ET AL: Storage of New Zealand jack mackerel (*Trachurus novaezelandiae*) in ice. J. Food Sci. (1984) 46(6): 1453.
6. SAITO, T. ET AL.: A new method for estimating the freshness of fish. Bull. Jap. Soc. Sci. Fisheries (1959) 24(9): 749.
7. SIMIDU, V.; HASUO, K.: An improved medium for the isolation of bacteria from marine fish. J. Gen. Microbiol. (1968) 52: 355.
8. SUMNER, J.L.; GORCZYCA, E.: Effect of vacuum packaging on the shelf life of fish held either in ice or at 4-6°C. IIF-IIR Commissions C-2, D-1, D-3 Boston, MA. (1981).
9. SCOTT, D.N. ET AL.: Storage characteristics of orange roughy held in ice. Fish Processing Bulletin (1984) 3, DSIR, Auckland.
10. VYNCKE, W.: Quality assessment of gutted and ungutted gurnard (*Trigla* spp.) by organoleptic and objective methods. Zeitschrift für Lebensmittel-Untersuchung und Forschung (1980) 171: 352.
11. HUSS, H.H.; ASENJO, I.: Storage life of gutted and ungutted white fish. Arsberetning (1976), Technol. Lab., Ministry of Fisheries, Denmark.
12. BUISSON, D.H. ET AL: Oil from deep water fish species as a substitute for sperm whale and jojoba oils. JAOCS (1982) 59(9): 390.
13. CASTELL, C.H.: Spoilage problems in fresh fish production. Bulletin 100 (1954), Fish. Res. Bd Can., Ottawa, Canada.
14. SHAW, S.J. ET AL.: Effect of delayed filleting on quality of cod flesh. J. Food Sci. (1984) 49: 979.
15. RATKOWSKY, D.A. ET AL: Relation between temperature and growth rate of bacterial cultures. J. Bacteriol. (1982) 149(1): 1.
16. OLLEY, J.: Temperature effects on histamine producing bacteria. In "Properties and Processing of Marine Foods" (1983), National Taiwan College of Marine Science and Technology, Marine Food Science Series.

CONSERVATION EN MAGASIN DE DETAIL DE HOPLOSTETHUS ATLANTICUS REFRIGERE - STRATEGIE DE RECHERCHE POUR UNE NOUVELLE PECHE.

RESUME : La stratégie de recherche adoptée pour Hoplostethus atlanticus est examinée et les résultats des expériences en matière de conservation en entrepôt et en magasin de détail sont présentés.

Dans un premier temps, un essai de conservation sur glace a mis en évidence les modifications physiques, chimiques, microbiologiques et sensorielles qui se sont manifestées durant la détérioration de poisson entier. Il est apparu que le goût de la chair cuite était le critère le plus important pour la détermination de l'acceptabilité du produit. La conservation en magasin de détail a été définie comme la période avant laquelle on note un goût et une odeur désagréables dans la chair cuite à la vapeur, soit 11-12 jours dans le cas de Hoplostethus atlanticus conservé sur glace.

Une série d'expériences a alors été effectuée pour étudier les variations de la conservation en magasin de détail dans des conditions diverses. Du poisson entier abîmé par le chalut avait une conservation en magasin de détail de 9-10 jours. Une réfrigération médiocre au départ représentait une perte de conservation égale à 6 jours sur glace. L'étêtage et l'éviscération prolongeaient la conservation sur glace de 2-3 jours, mais ces opérations n'étaient pas jugées commercialement opportunes. Les filets frais avaient une durée de conservation de 5-7 jours à 0°C. A condition d'utiliser du poisson dans un état suffisamment bon, la qualité du poisson entier avait peu d'influence sur la conservation en magasin de détail des filets prélevés. Une double congélation/décongélation a causé une perte de conservation de 2-3 jours par rapport à une congélation unique. La conservation de filets décongelés et conservés à des températures différentes est conforme à la relation Olley-Ratkowsky.

DISCUSSION

K.J. WHITTLE (U.K.) - 1) What is the age distribution in the catch of orange roughy ?

2) Can you say what catch the stock will sustain ?

D.N. SCOTT - 1) Studies have shown that Hoplostethus atlanticus is a slow growing fish. Uncertainty about the stock size and age distribution have therefore led biologists to set fairly conservative stock assessments until more research has been carried out.

2) There are many different opinions as to what the stock can sustain. The current allowable catch is 38,500 tonnes but the industry maintains this can be higher. Current research includes exploration of other grounds to determine if there are other commercial quantities of orange roughy within the EEZ.

W. SCHREIBER (FRG) - 1) What is the fat content of orange roughy fillets ?

2) Did you conduct experiments with frozen fish ?

D.N. SCOTT - 1) Filleting is followed by deep skinning thus most of the subcutaneous layer is removed. This process results in a fillet with an oil content of about 9.9%.

2) Storage trials on frozen orange roughy were abandoned due to freezer breakdowns. The fish, however, have been kept in good condition for one year at -30°C.

CONSUMER ACCEPTABILITY AS THE BASIS FOR DETERMINING
THE CHILLED SHELF LIVES OF SMOKED PRODUCTS
FROM THAWED HERRING AND MACKEREL

P. D. BAIRD, R. BENNETT and M. HAMILTON
Robert Gordon's Institute of Technology, Aberdeen
J. G. M. SMITH and K. J. WHITTLE
Torry Research Station, Aberdeen (United Kingdom)

1. INTRODUCTION

Consumer awareness of oily fish species is low in the UK and less than 20% of the potential catch is consumed. Whittle /1/ suggested that in the light of the rapid decline in landings of the more popular demersal fish such as cod and haddock, greater exploitation of underused species such as mackerel and herring represented a major means of expanding the domestic industry.

Alongside the sales of oily fish in the whole form there has been a long standing delicatessen market for cured products, such as kippered and soured herring, smoked mackerel and various types of pâté. Expansion of this market and that for canned products offer further alternatives for increasing sales.

Connell and Hardy /2/ suggested that the UK consumer had suspicions about the quality and freshness of fish products. Wesson *et al.* /3/ reported that the fish industry in the USA was reluctant to implement expensive storage methods to ensure quality and freshness because of a lack of evidence of the consumer's ability to assess fish quality. They then established that the American consumer could detect oxidised flavours and preferred the fresh product.

There have been a number of studies of the changes during storage of demersal species under both chilled /4/ and frozen conditions /5, 6/ and of consumer reaction /7/ to the changes. However, relatively little is known of attitudes towards storage changes in oily fish. It is well known that the effects of spoilage are due largely to autolytic enzyme activity and to bacterial action. Oily fish are not usually gutted before sale. Autolysis in the gut can be rapid particularly if temperatures are high and the fish had been feeding actively before capture. Additionally, the oil content is readily oxidised to produce a characteristic rancidity. Clearly, the changes occurring in oily fish during chilled storage, and consumer reaction to them, are of considerable importance in relation to potential market expansion.

The first stage of the study of these factors, described below, examines responses to products prepared from whole fish which previously had been cold stored, since much of the herring and mackerel processed in the UK at present is handled in this way.

2. MATERIALS AND METHODS

2.1 Fish samples

2.1.1 Supply: The mackerel were caught in October, 1984 by the stern trawler G A Reay, frozen whole immediately after hauling in 25 kilo blocks in polythene bags topped up with water to protect against dehydration and oxidation, and stored at -30°C for 12 weeks. Blocks were thawed overnight at ambient temperature before use.

The herring were caught, handled and frozen as above, and stored at -30°C for 5 months prior to thawing as above. During subsequent chilled storage of whole fish and smoked products, the temperature of samples remained between 0°C and -0.4°C.

2.1.2 Unsmoked samples: Whole herring and mackerel were stored well iced in a chillroom (2-4°C). At various intervals, fish were removed, block filleted, vacuum packed and stored at -30°C until required for tasting within the next 2 weeks. These samples are termed 'unsmoked' in the text and were presented to panellists as cooked samples.

2.1.3 Hot smoked mackerel cutlets: Samples of freshly thawed whole mackerel were block filleted and held in 60° brine solution (3M) for 3 minutes and drained in the chillroom until hot smoked the following day in the Torry mechanical smoking kiln for 1 hour at 27°C, 1 hour at 49°C and 45 minutes at 71°C. After cooling the cutlets were stored for 10 days as pairs, flesh to flesh, between thin polyethylene sheeting and were well iced. Melt water could not reach the samples and draining and reicing were carried out every third day. Samples were removed at intervals, vacuum packed and stored at -30°C until required. They are termed 'smoked mackerel' below and were presented to panellists without further cooking.

2.1.4 Kipperd 'boneless' herring cutlets: Thawed herring were block filleted, brined and drained as above. The cutlets were smoked for 2 h 20 min at 27°C and stored well chilled as above. Periodically, kippers were removed, packed, frozen and stored as above. These samples were cooked before presentation to the panellists and are termed 'smoked herring' below.

2.2 Preparation of samples for tasting

Cooked samples were prepared by wrapping frozen fillets in aluminium foil and baking at 180°C for 20 min. The hot smoked mackerel cutlets were thawed at room temperature overnight. No condiments were used during cooking or provided at tasting sessions.

2.3 Presentation of samples

The tail end of each fillet was removed and the whole fillet served on identical, coded, disposable, white polystyrene plates. Tasting was carried out in individual booths and tepid water was provided for rinsing the mouth.

2.4 Sensory evaluation

Forty-two assessors comprising academic, secretarial and ancillary staff, and home economics students were used. The panel was 80% female and none of the panellists had been trained in fish tasting. Samples were scored using a 9-point hedonic rating scale (9 = like extremely; 5 = neither like nor dislike; 1 = dislike extremely). Only unit marks were allowed and assessors rated the samples for appearance, texture, flavour and overall acceptability. Samples were presented in random order and each panellist assessed up to 4 samples at a tasting session.

Preliminary sensory assessment was also carried out on first and final day samples by 3 assessors experienced in judging oxidative spoilage in oily fish species.

2.5 Statistical analysis

The distribution of hedonic panel ratings usually showed a marked degree of skewness and so, instead of the normal summary statistics of mean and standard deviation, the calculated median values were used as a measure of the centre of the distribution. Lines of best fit were calculated by the method of least absolute deviations /8, 9/, which is the regression analogue of the median and is less influenced by outlying or aberrant values than the usual method of "least squares" as discussed by Smith /10/.

Percentage liking represents those panellists who expressed any degree of "liking", i.e. the number of scores 6 to 9 plus half the scores 5.

2.6 Chemical analysis

Chemical analyses were carried out on the zero and final day samples. Total lipid content was determined using a solvent extraction method on homogenised samples after addition of antioxidant (Bligh and Dyer /11/ as modified in Torry Research Station Advisory Note 89 /12/). Peroxide values (PV) of the extracted lipid were determined /13/ and salt /14, 12/ and histamine /15/ concentrations determined.

3. RESULTS AND DISCUSSION

TABLE I

Results of chemical analyses carried out on unsmoked fish and smoked cutlets for the first and final days of sampling

	MACKEREL				HERRING			
	Unsmoked		Smoked		Unsmoked		Smoked	
	0 Day	11 Day	0 Day	10 Day	0 Day	11 Day	0 Day	15 Day
Salt content as a % of water content	0.7	0.6	4.0	4.1	0.8	0.8	3.5	3.9
Percentage Lipid	19.6	18.9	19.1	20.3	7.9	11.0	9.5	8.2
Peroxide Value *	1.5	11.3	1.1	5.1	1.8	28.8	4.0	7.5
Histamine .mg/100 g tissue		0.8		0.5		0.2		0.5

* ml 0.002N sodium thiosulphate/g lipid

Table I shows the results of the chemical analyses. A PV of 10 corresponds reasonably well with the nearest point at which rancid flavours were detected by Smith *et al.* /16/; the final day unsmoked products exceeded that value. Expert assessors noted very slight rancidity in the brown flesh of 15 day smoked herring and 10 day smoked mackerel, definite rancid flavours in the brown flesh and slight rancid flavours in the white flesh of 11 day unsmoked mackerel, and strong rancid and stale flavours in 11 day unsmoked herring. All zero time samples were found to be acceptable by expert assessors and the low PV shows that the recommended frozen storage conditions Smith *et al.* /17/; Hardy *et al.* /18/ used for the whole fish prior to processing, were satisfactory. The low histamine values obtained for all final day samples show that the chilled storage procedures had been effective and probably also indicate that the histamine producing bacterial flora had been depleted during the initial freezing and frozen storage of the whole fish after catching.

Figure 1 shows the medians of the distribution of acceptability ratings of smoked and unsmoked mackerel against chilled storage time and Figure 2 shows the same for smoked and unsmoked herring.

Figure 3 shows the percentage of panellists expressing some degree of liking for smoked and unsmoked mackerel against storage time and Figure 4 shows the same for smoked and unsmoked herring. The data are plotted with the regression lines superimposed.

Unsmoked mackerel was very acceptable at zero time and its acceptability diminished progressively with chilled storage. The decrease in acceptability was statistically significant after 4 days storage.

Smoked mackerel was rated more highly than unsmoked and its acceptability declined more slowly during storage. The decrease in acceptability was statistically significant after 8 days storage. In view of these findings, the storage trials with smoked herring were extended to 15 days.

Unsmoked herring was only just acceptable initially to assessors and its acceptability declined progressively at about the same rate as unsmoked mackerel but from a much lower starting point. The decline in acceptability was again statistically significant after 4 days. The expert judges had noted that zero time herring, although acceptable, lacked the normal fresh herring flavour. It was noted also that the fillets progressively developed blood stains in the belly wall area

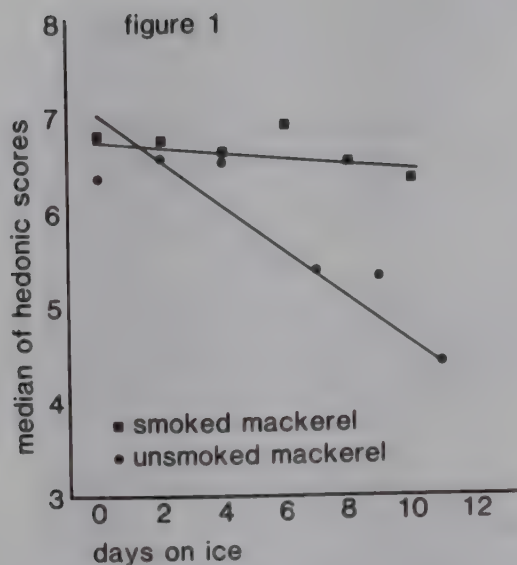


Fig. 1 - Change in median hedonic scores during iced storage of whole mackerel and hot smoked mackerel cutlets.

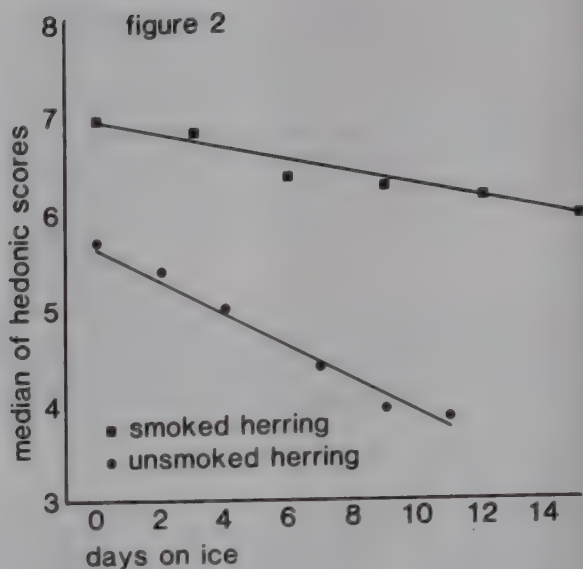


Fig. 2 - Change in median hedonic scores during iced storage of whole herring and "kippered" herring cutlets.

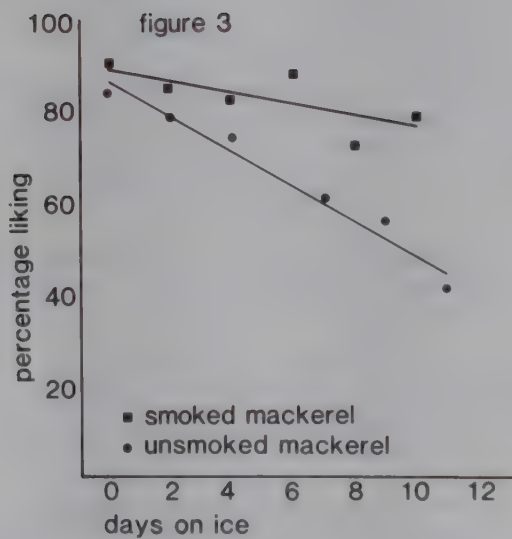


Fig. 3 - Change in percentage liking during iced storage of the mackerel samples shown in Fig. 1.

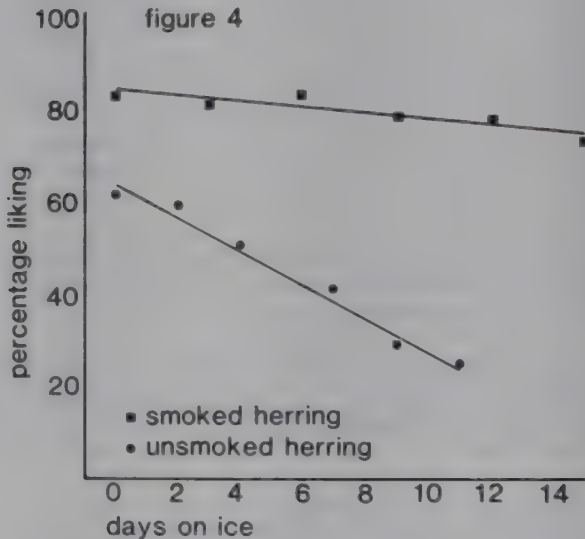


Fig. 4 - Change in percentage liking during iced storage of the herring samples shown in Fig. 2.

during storage. The subjective view was that this was a batch of fish of low intrinsic quality; the loss in quality did not seem to be connected with the manner of handling or frozen storage.

Smoking the herring raised its acceptability substantially and the decline in acceptability with time of the smoked product was again at a reduced rate and similar to that of the hot smoked mackerel. The decline in acceptability was statistically significant after about 9 days.

Figures 1 and 2 show that the decline in acceptability scores with time is remarkably linear over the observed time scale, particularly since assessors were rating only with unit scores. The same pattern has been observed with cod and whiting by Bennett and Hamilton, /19/ and with haddock by Connell and Howgate, /7/.

The general consensus is that loss of acceptability in fresh fish is initially attributable to a loss of some valued, characteristic and intrinsic fresh flavour and, subsequently, to the development of disliked flavours by autolytic and oxidative reactions and by bacterial action and, to softening of texture. The process is clearly multiphasic and, since bacterial growth is exponential rather than linear, it is not at all obvious how these processes produce the linear pattern observed. In the case of the smoked products, smoking clearly confers a liked dimension; the process of brining and smoking either aids preservation or the introduced taste and flavour masks the development of off flavours (or possibly both). In this context, it should be noted that the rates of loss of acceptability of the hot smoked mackerel and of the cold smoked herring are very similar despite the fact that it might be anticipated that the process of hot smoking would have a greater preservative action as a result of the higher salt content (Table I), the longer smoking time and the higher temperatures.

Manufacturers and retailers have to make recommendations and decisions regarding the chilled shelf life of products. This is clearly a complex matter which is of considerable commercial importance. The acceptability of foodstuffs is subject to many influences and individuals respond differently. Thus, in our study, not all assessors liked the freshest product and not all assessors disliked the stored product. Howgate /20/ advanced ideas for decision making in this area, and Figures 3 and 4 show, as he suggests, plots of the percentage of judges who express some degree of liking for the product against storage time. The decision regarding the level at which to set chilled shelf life is arbitrary. However, if 70% is selected as the cut-off point, then the smoked products have a shelf life at 0°C of more than 10 and possibly 15 to 16 days. Unsmoked mackerel was about 4 days but the unsmoked herring in the trials would never be sold. It is likely that these shelf lives at 0°C are conservative since the acceptability of the products would not be under such detailed scrutiny in domestic circumstances, and the addition of condiments, sauces and accompaniments would almost certainly raise the general levels of acceptability. This effect has been demonstrated in work by Hamilton and Bennett /21/ using whiting and blue whiting.

REFERENCES

1. K. J. WHITTLE (1983). IFST Proceedings. 17, 2, 59.
2. J. J. CONNELL and R. HARDY (1982). Trends in Fish Utilisation. Fishing News (Books) Ltd, Surrey, England, pp. 44-46, 93.
3. J. B. WESSON, R. C. LINDSAY and D. A. STUIBER (1979). J. Fd Sci., 44, 878.
4. J. M. SHEWAN, R. G. MACINTOSH, C. G. TUCKER and A. S. C. Ehrenberg (1953). J. Sci. Fd Agric., 4, 283.
5. J. J. CONNELL and P. F. HOWGATE (1968). J. Sci. Fd Agric., 19, 342.
6. J. J. CONNELL and P. F. HOWGATE (1969). Ibid 20, 469.
7. J. J. CONNELL and P. F. HOWGATE (1971). In 'Fish Inspection and Quality Control' Ed. R. Kreuzer pp. 155-159 Fishing News (Books) Ltd, Surrey, England.
8. O. J. KARST (1958). J. Am. Statist. Soc., 53, 118.
9. R. D. ARMSTRONG and M. T. KUNG (1978). Applied Statistics 27, 363.
10. G. L. SMITH (1984). In 'Sensory Analysis of Foods' Ed. J. R. Piggott pp. 305-349. Elsevier Applied Science Publications Ltd, London.
11. E. G. BLIGH and W. G. DYER (1959). Can. J. Biochem. Physiol., 37, 911.
12. A. AITKEN, A. LEES and J. G. M. SMITH Torrey Research Station Advisory Note No. 89. 'Measuring Fish Composition'.
13. A. BANKS (1937). J. Soc. Chem. Lond., 56, 13J.

14. W. HORWITZ (1980). Official Methods of Analysis of the Association of Official Analytical Chemists. 13th Edition p. 289 AOAC Washington.
15. S. L. TAYLOR, E. R. LICHER and M. LEATHERWOOD (1978) J. Fd Sci., 43, 247.
16. J. G. M. SMITH, R. HARDY, I. MCDONALD and J. TEMPLETON (1980a). J. Sci. Fd Agric., 31, 375.
17. J. G. M. SMITH, A. S. MCGILL, A. B. THOMSON and R. HARDY (1980b). In 'Advances in Fish Science and Technology' Ed. J. J. Connell, Fishing News (Books) Ltd, Surrey, England p. 303.
18. R. HARDY, J. G. M. SMITH and K. W. YOUNG (1973). 'Conferences in Conjunction with the 8th International Exhibition for the Food and Allied Industries', London. BPS Exhibitions Ltd, pp. 1-5.
19. R. BENNETT and M. HAMILTON (1985). J. Fd Technol. (In Press).
20. P. F. HOWGATE (1983) Proceedings of the International Institute of Refrigeration XVI Congress, Paris (In Press).
21. M. HAMILTON and R. BENNETT (1981). J. Fd Technol., 16, 655-659.

TEST DE CONSOMMATION COMME BASE DE LA DETERMINATION DE L'APTITUDE A L'ENTRE-POSAGE PAR REFRIGERATION DES PRODUITS FUMES, PREPARES AVEC DES HARENGS ET MAQUEREUX DECONGELES.

RESUME : Les maquereaux et harengs entreposés à -30°C ont été décongelés. Les maquereaux ont été réfrigérés entiers puis les filets ont été fumés à chaud sous forme de bloc pendant respectivement 11 et 10 jours; les harengs ont été réfrigérés entiers puis les filets ont été fumés à froid pendant respectivement 11 et 15 jours. On a déterminé leur valeur en peroxyde au début et à la fin de la période d'entreposage ; la qualité s'est dégradée progressivement dans tous les échantillons mais de façon nettement plus lente dans les produits fumés.

Les échantillons ont été cuits, si nécessaire, et ont été jugés du point de vue de leur aspect, texture, flaveur et qualité générale par 40 personnes non entraînées en appliquant l'échelle hédonique à 9 points. Le score moyen des maquereaux non fumés a baissé progressivement et les échantillons au temps zéro ont été préférés de façon significative à ceux stockés pendant 7 - 9 et 11 jours. Le score moyen des maquereaux fumés à chaud a diminué beaucoup plus lentement et des différences significatives n'ont été trouvées qu'entre les échantillons au temps 8 et 10 jours par rapport aux échantillons au temps zéro. Si la durée maximale d'entreposage est celle au bout de laquelle moins de 70 % des dégustateurs considèrent le produit comme acceptable, la durée de conservation par réfrigération des maquereaux décongelés crus est d'environ 4 jours et celle des maquereaux fumés à chaud plus de 10 jours.

Les résultats sont comparés avec ceux de recherches similaires sur des harengs.

DISCUSSION

H.H. HUSS (Denmark) - There is a big difference between the reactions of your consumer panel and the expert panel of Torry Research Station as presented by Miss Hendrie in her paper. Would you care to comment on this ?

R. BENNETT - Since this paper deals with an unpacked product and Miss Hendrie's work was concerned with a vacuum packed product it is difficult to make an exact comparison. However, the major point is that our consumer panel simply expressed a degree of liking for the samples whereas the expert panel were scoring on a freshness scale. The paper presented by I Mackie on storage trials clearly shows that trained panels detect and score changes which are neither noticed by consumer panels or just do not influence their liking for the product.

STORAGE CHARACTERISTICS OF ICED WHOLE LOLIGO SQUID

J. J. LICCIARDELLO, E. M. RAVESI, S. M. GEROW
AND D. D'ENTREMONT

National Marine Fisheries Service, Northeast
Fisheries Center, Gloucester Laboratory,
Emerson Avenue, Gloucester, Ma 01930 (U.S.A.)

INTRODUCTION

The abundant squid resources of the Northwest Atlantic offer the potential for a substantial, sustained fishery for North American fishermen /1, 2/; however, this potential has not yet been fully realized although the fishery is expanding. The average yearly U. S. commercial landings along the Atlantic coast for the five year period 1974-1978 were 5.5 million pounds (2494 metric tons) /3/, but the catch has since been steadily increasing and 17.4 million pounds (7891 tons) were landed in 1982 and 33.5 million pounds (15193 tons) in 1983 /4/. There are two species of commercial importance along the U. S. Atlantic seaboard: Loligo pealei, the long finned or "bone" squid is most commonly found from Cape Cod to Cape Hatteras although its range extends from Venezuela to Nova Scotia; Illex illecebrosus, the short-finned or "summer" squid is most abundant in the waters from the Maritime Provinces in Canada to Cape Cod /5/. Of the two species Loligo is the preferred because of its more desirable sensory characteristics (flavor, texture, whiteness). Although there has been a slight increase in the domestic squid market in recent years, the increased landings are principally due to newly found export opportunities and joint ventures with foreign vessels. In dealing with export markets certain quality criteria or specifications are usually imposed by the importer. Upon reviewing the scientific literature we could find no information relating quality of fresh Loligo squid with chemical or physical indices usually written into quality specifications, although some studies had been carried out with Illex at the Department of Fisheries and Oceans, Halifax, Nova Scotia. Therefore, the present investigation was undertaken to determine storage changes in iced Loligo pealei and to evaluate the feasibility of some commonly used objective tests for assessing quality or spoilage.

MATERIALS AND METHODS

Trawl-caught squid (Loligo pealei) were boxed at sea in standard wood fish boxes of 125 pound (57 kg) capacity, received at the Gloucester Laboratory the following day, repacked in flake ice (2 parts ice/1 part squid), and put into a cold room at approximately 35°F (1.7°C). The squid were considered of two days post mortem age at the beginning of the storage study.

At various time intervals, 12 randomly selected squid were examined in the raw state for appearance (skin color and condition of eyes), odor and color of flesh. Six separate readings were taken on each squid with a Torrymeter on the central area of the tube with care exercised to avoid measurements on damaged skin. The six replicate readings were averaged to obtain the mean for each sample and the grand average represented the Torrymeter reading for that storage time.

The 12 squid were then cleaned, skinned and composited by fine chopping and mixing. Only the mantles were used. Portions of the composite sample were analyzed for the following:

Ammonia. Ammonia (NH_3) content was determined by the microdiffusion method developed by Seligson and Seligson /6/ and later modified by Vyncke /7/.

DMA, TMA, TMAO. Dimethylamine (DMA) and trimethylamine (TMA) were determined by a gas chromatograph method described by Tokunaga et al /8/ and modified by Lundstrom and Racicot /9/. Modification included substitution of a 9 foot x 2 mm i.d. glass column packed with Chromosorb 103, which allowed a baseline separation of DMA and TMA, and use of a nitrogen-phosphorous specific flame ionization detector. For quantification of the amines, N-propylamine was employed as an internal standard. Trimethylamine oxide (TMAO) was calculated as TMA after reduction by titanous chloride and determined by difference /10/.

I.I.F. - I.I.R. Commissions C₂, D₃ - Aberdeen - (United Kingdom) - 1985-4

ph. A 20 gram sample was blended with 40 ml distilled water for one minute and the pH of the slurry was measured with a Fisher* Model 320 expanded scale pH meter.

Hypoxanthine. The enzymatic procedure described by Jones et al /11/ was followed for the assessment of hypoxanthine content.

Aerobic plate count. The aerobic plate count (APC) was made from appropriate phosphate-buffered decimal dilutions onto pour plates of Standard Methods Agar reinforced with 0.5 percent Bacto-peptone and 0.5 percent NaCl as recommended by Lee and Pfeifer /12/ for seafoods. Duplicate plates were incubated at 68°F (20°C) and colony counts made after five days.

Color. A portion of the composite sample was comminuted for two 30 second periods in a Robot Coupe Model R2 Commercial Food Processor. The homogenized sample was packed into a 100 x 15 mm plastic petri dish and color was measured by tristimulus Y, X, Z values (L, a, b-type scale) using a Hunter Lab Color/Difference Meter Model D25-2.

Sensory analysis. Three squid were used for organoleptic evaluation. The skinned mantles were cut into strips about 3/4 x 2 inches (20 x 50 mm), enclosed in foil and steamed for 15 minutes. A laboratory panel of six members experienced in tasting squid of variable quality was instructed to examine a strip from each of the three squid and to score as a single sample for appearance, flavor and texture on a scale of 1 to 9 where 9 = excellent, 8 = very good, 7 = good, 6 = fair, 5 = borderline, 4 = sl. poor 1 = inedible. Shelf life was considered to have expired when the average score for any of the sensory attributes fell below 5.5.

Statistical methods. Assays for chemical, physical or microbiological parameters were performed in duplicate and results averaged. Statistical analysis for linear regression, correlation, etc. were conducted on a Hewlett-Packard 97 programmed calculator.

RESULTS AND DISCUSSION

At the start of the storage study the squid were considered of excellent quality. The eyes were clear, there was little or no damage to the skin which was distinctly speckled with no fading or running of the color, the flesh was white and the body contents were odorless. The eyes maintained their clarity for only a few days being judged moderately cloudy on the fifth day and definitely cloudy on the ninth. Other than some random ink stains the skin remained in good condition through day five. Distinct pigment spots due to coalescence were evident on the seventh day and there was a progressive deterioration to severe discoloration, soft and torn skin by day fourteen. Some transfer of skin pigment to the flesh was observed on the seventh day, it became more noticeable on the ninth, and by day eleven there was considerable pigmentation to the flesh on the skin side, and discoloration from ink and visceral organs on the inside. The odor of the viscera remained good through nine days, but signs of incipient spoilage were detected on the eleventh day. Odor was judged poor on the fourteenth day.

The decline in sensory score of various attributes of cooked squid during iced storage is shown in Fig. 1.

The shelf life was estimated as 11 days. Deterioration in appearance was the limiting quality factor. A slight discoloration of the cooked flesh was observed after seven days post mortem and this became more apparent after nine days. On the twelfth day there was a marked pink-yellow discoloration which by the fourteenth day had turned to various shades of yellow-purple-black. The progressive color changes as monitored with the Hunter Lab Color Meter are shown in Fig. 2.

Takahashi /13/ has stated that as spoilage of squid progresses, the skin pigment cells decompose and release the pigment which reddens the flesh. He suggested that freshness of squid could be approximated by the change in skin color. The yellow-brown stain that develops on the inside of the mantle is reputed to be caused by leakage from the liver /14/. This latter form of deterioration is more prevalent in

*Mention of trade names does not constitute endorsement by National Marine Fisheries Service, NOAA.

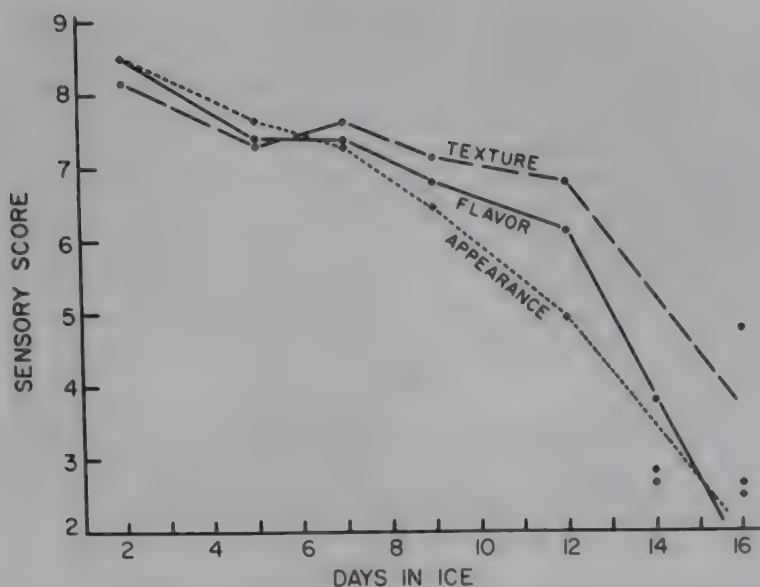


Fig. 1. Sensory score of Loligo squid during iced storage.

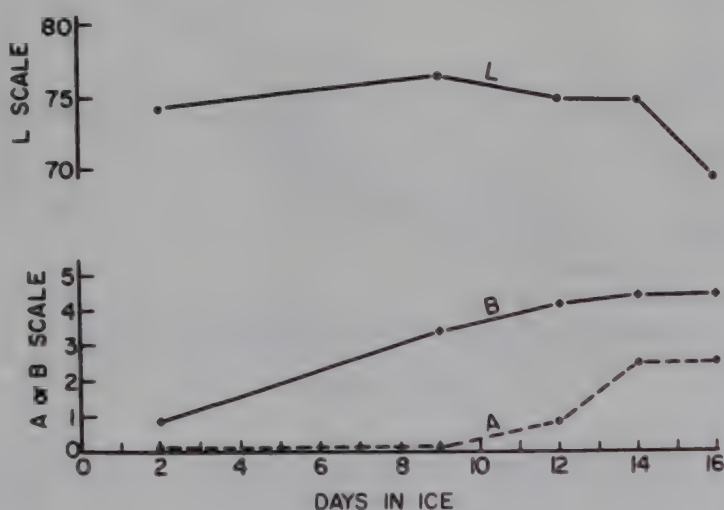


Fig. 2. Hunter Colorimeter values of Loligo squid flesh during iced storage.

squid that are crushed during transport. Ke et al /15/ developed a guide for grading whole squid (*Illex*) based on color of skin and flesh, odor and texture. In accordance with the overall quality score the product would be rated either A (excellent - good), B (fair - acceptable) or F (unacceptable - spoiled). It was pointed out that much processed squid is sold with skin removed in which case eating quality would have to be judged principally on the merit of flavor and texture. It is not recommended that quality of squid be judged on skin color alone since that property can be manipulated by storage methods /16/. The skin color is controlled by muscles attached to the chromatophore cells. As quality deteriorates, the muscles relax and the color becomes white. The desirable dark brown skin coloration was also said to depend on availability of oxygen, storage temperature and non-contact with freshwater. Certain ions in water contacting squid were also reported to affect color /17/. Botta et al /18/ estimated the storage life of squid (*Illex*) in freshwater ice as three days based on skin color, and eight and one-half days based on sensory

evaluation of the cooked fish. These researchers concluded that skin color was not necessarily related to eating quality. They also found that storage of squid in either refrigerated seawater, circulating chilled seawater or in non-contact fresh-water ice was more effective in preserving color and eating quality compared to holding in non-circulating chilled seawater, salt water ice or in contact with freshwater ice. Published values for iced shelf life of squid based on sensory evaluation show some variability among different investigators. In the present study the estimated storage life was 11-12 days and this is to be compared to values reported by others of 5, 5-10, 8.5, 9 and 13 days /21, 5, 18, 19, 20/. Part of this discrepancy might be explained by differences in quality criteria used in defining acceptability or nonacceptability. Other variable factors include squid to ice ratio, method of stowage (boxed, penned, etc.) /19/, season of year /5, 22/ and mode of capture (trawling, jigging, etc.) /23/.

Through seven days post-mortem iced storage there was no significant (5% level) change in either flavor or texture, but a change was observed on the ninth day. This would agree with the finding of Stroud /20/ who reported that iced whole squid keep in first class condition for up to eight days.

Ammonia content of the flesh increased slowly during the first nine days and rapidly thereafter (Fig. 3).

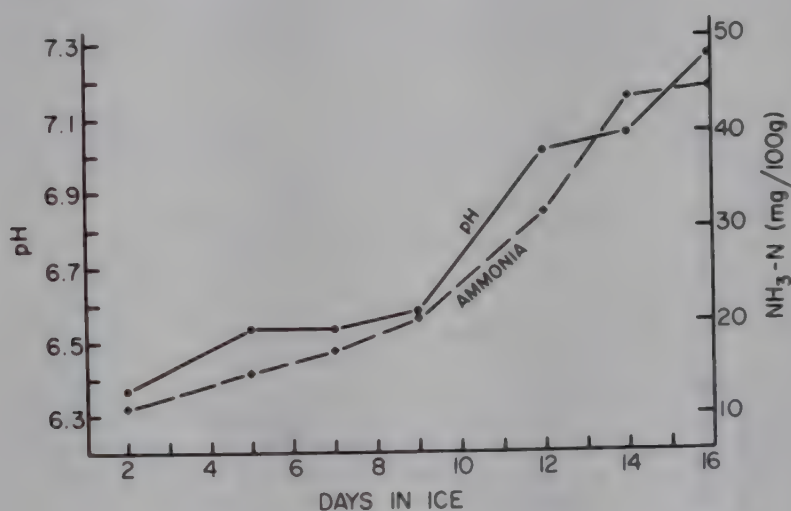


Fig. 3. Ammonia content and pH of *Loligo* squid during iced storage.

When plotted on semi log paper a linear (first order) rate was indicated. Flesh pH followed a similar pattern (Fig. 3), as might be expected, since the ammonia content would exert a strong influence on that parameter. There was excellent correlation ($r = 0.97$, $n = 7$) between ammonia content and flavor score. From regression analysis a flavor score of 5.5, representing marginal quality, was found to predict an ammonia content of 29 mg % - N with 95 percent confidence limits of 25 - 32. Mantle pH also correlated well ($r = 0.90$, $n = 7$) with flavor score and at incipient spoilage a pH of 6.8 was estimated with confidence limits of 6.67 - 6.97. Mustiness in raw squid, which has been interpreted as a sign of spoilage, was observed at pH > 6.5 /24/. From past experience with certain finfish we have found that muscle pH usually correlates well with flavor score in individual iced studies, but this parameter is not usually reliable as a spoilage indicator when applied to different batches of fish because of excessive variability.

The accumulation of volatile amines during storage can be seen in Fig. 4.

DMA-N remained relatively constant over the 16 day post-mortem period with an average content of 3.68 mg % (0.26 mM DMA/100g). This parameter would therefore have no utility as a spoilage indicator in fresh squid. Tokunaga et al /25/ observed an approximate doubling of DMA content in squid from about 0.4 to 0.7 mM during six days

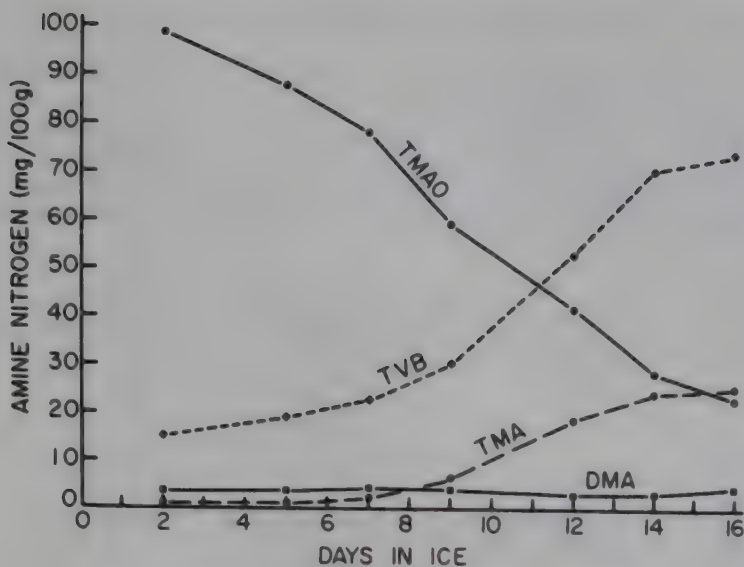


Fig.4. Volatile amine content of Loligo squid during iced storage.

at room temperature, however, the species and temperature were both dissimilar to that used in the present study. In frozen stored squid DMA is produced from enzymatic degradation of TMAO and its content is related to textural quality /26/ and not to pre-freezing bacterial activity.

TMA remained at an insignificant level for about seven days and then increased steadily reaching a value of 25.7 mg % N after 16 days. LeBlanc and Gill /27/ also reported no practical change in TMA content of *Illex* squid during the first five to six days at 36.5°F (2.5°C) and for that reason concluded that TMA would be an unreliable index of squid quality. From linear regression of TMA content on flavor score ($r = 0.93$), a value of 13 mg % TMA-N with confidence limits of 8.7 - 17.7 was predicted at spoilage. Woyewoda and Ke /16/ recommended the following quality guidelines for *Illex* squid based on TMA-N content: < 3 mg % = excellent, 3-10 = acceptable, >10 = unacceptable. Slabyj and True /21/ reporting on a storage study with whole *Illex* squid stated that borderline samples contained about 50 mg % TMA-N. The following quality grades based on TMA content were specified by a foreign interest in negotiations with the Mid Atlantic Fisheries Council for a joint venture in squid: excellent = 0 - 0.75 mg %, good = 0.75 - 1.5 mg %, average = 1.5 - 3.0 mg %, bad = 3.0 - 5.0 mg % and putrid = >5 mg %. There appears to be some disparity in TMA standards for squid quality. Some of this disagreement may be due to the methodology employed and perhaps the TMA specification for squid quality should include the method to be employed. Inaccuracies associated with various TMA procedures have been pointed out by Shewan et al /28/. In the present study the squid were regarded to be of excellent - very good eating quality up to a TMA-N content of 6 mg % and acceptable up to 13 mg %.

Total volatile base content is probably the most commonly applied chemical index for assessing squid quality. A graph of TVB accumulation was constructed by plotting the sum of the contents of TMA, DMA and ammonia for each storage time (Fig. 4). Flavor score and TVB correlated very well ($r = 0.96$) and the predicted value for borderline acceptance from regression analysis was 45.7 mg % N (confidence limits = 38-53). Up to a value of 30 mg % N the eating quality was regarded as excellent - very good. These results for *Loligo* do not agree with some reported for other species. Tanikawa et al /24/ detected musty flavor in raw squid when the TVB-N reached 30 mg % and this value was proposed as the index for the first stage of putrefaction /29/. In a study with *Illex* squid held at 36.5°F (2.5°C) the TVB-N content was about 30 mg % when samples were judged to be spoiled /27/. Our results were in excellent agreement with quality guidelines recommended by Woyewoda and Ke /16/ as follows: <30 mg % = excellent, 30-45 = acceptable, >45 = unacceptable. The variable results among different researchers may be partially explained by differences in discrimination as to what constitutes spoilage, however, there can be substantial discrepancies

in TVB results depending upon the analytical method used /30/.

TMAO content decreased at a linear rate dropping from a value of about 100 mg % N at two days post mortem to approximately 24 at 16 days (Fig. 4). The steady decline (6 mg % N/day) of this parameter from the start presents the potential for an effective quality test, however, the reliability of this indicator would have to be proven by determining the degree of variability among individual squid. TMA and DMA are derived from TMAO, yet it is interesting to note the lack of a material balance at any test period.

During the first 11 days post mortem there was just a slight increase in hypoxanthine content, but beyond that time the production rate increased sharply (Fig. 5).

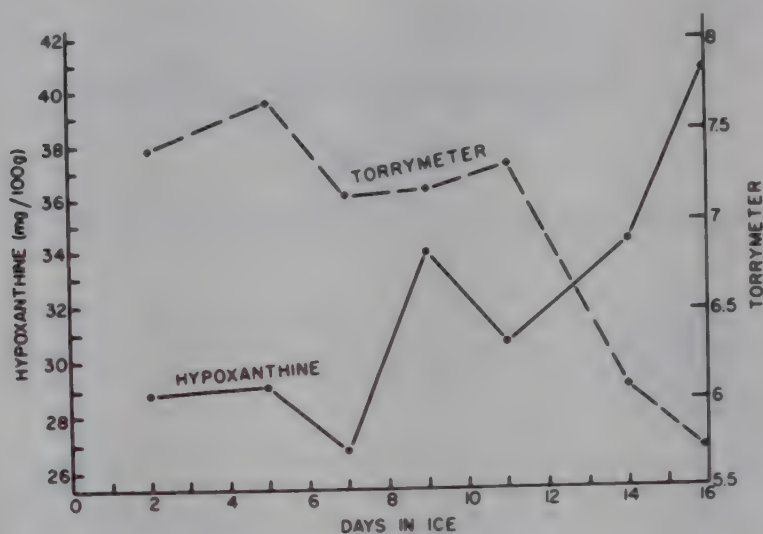


Fig. 5. Hypoxanthine content and Torrymeter reading of *Loligo* squid during iced storage.

The estimated quantity at spoilage was 32.8 mg % ($2.4 \mu\text{M/g}$). This is about the same as reported for cod at rejection /31/. Although there was good correlation with flavor score ($r = 0.82$, $n = 7$), it is not considered that this parameter would be useful as a quality indicator because of the very slight change during useful shelf life. A significant increase occurred only when the samples were badly decomposed.

The average Torrymeter reading of 12 squid per sampling decreased slightly during the first 11 days and then dropped precipitously. For this reason the instrument would probably not find use for measuring early quality changes, but could have application for assessing spoilage. It is essential that multiple readings be taken on each squid and that a large number are sampled. The standard deviation for the means of 12 squid was 0.74 and the mean standard deviation for six replicate readings on each squid was about the same. Average reading correlated well ($r = 0.94$) with flavor score and the estimated average value at spoilage was 6.8.

Aerobic plate count only changed slightly during the first seven days post mortem, but then increased repaidly as the cells entered the logarithmic phase of growth (Fig. 6).

The rise in count correlated very well ($r = 0.95$) with the drop in flavor score and an APC of 1.5×10^6 was estimated at incipient spoilage. LeBlanc and Gill /27/ only detected low numbers of bacteria on stored squid and they attributed their observed low TMA production to this event. From past experience with finfish we have found that at spoilage the APC is of the order of $10^6 - 10^7$, which agrees with the squid results; however, because of the variability in spoilage level from one study to another this parameter is not generally considered a reliable indicator of quality.

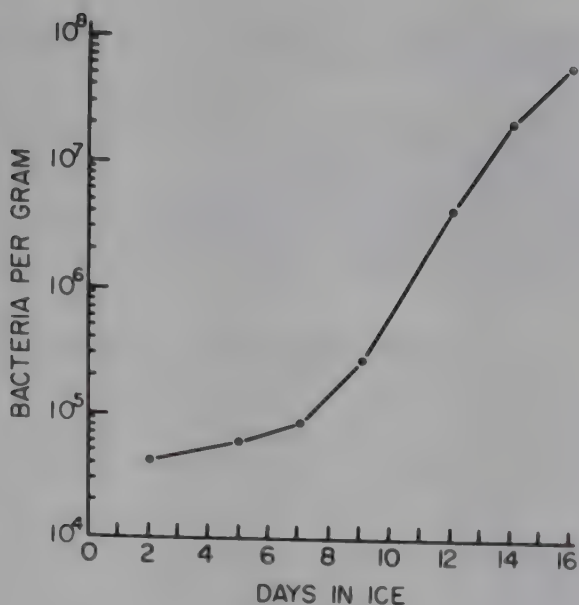


Fig. 6. Aerobic plate count of *Loligo* squid during iced storage.

REFERENCES

1. W. F. Rathjen: Northwest Atlantic squids. *Mar. Fish. Rev.*, Vol. 35 No. 12, (1973), pp. 20-26.
2. F. E. Lux, W. D. Handwork, and W. F. Rathjen: The potential for an offshore squid fishery in New England. *Mar. Fish. Rev.*, Vol. 36 No. 12, (1974), pp. 24-27.
3. B. G. Thompson: U. S. commercial landings. *Fisheries of the United States*, 1979. U. S. Department of Commerce, NOAA/NMFS, Wash., D. C., (1980), pp. 3.
4. B. G. Thompson: U. S. commercial landings. *Fisheries of the United States*, 1983. U. S. Department of Commerce, NOAA/NMFS, Wash., D. C., (1984), pp. 3.
5. V. G. Ampola: Squid - its potential and status as a U. S. food resource. *Mar. Fish. Rev.*, Vol. 36 No. 12, (1974), pp. 28-32.
6. D. Seligson and H. Seligson: A microdiffusion method for the determination of nitrogen liberated as ammonia. *J. Lab. Clin. Med.*, Vol. 38 (1951), pp. 324-330.
7. W. Vyncke: The influence of temperature on fish. *Fish. News Int.*, Vol. 7 (1968), pp. 49-50, 53.
8. T. Tokunaga, H. Iida, and K. Mirva: The gas chromatographic analysis of amines in fish. *Bull. Jap. Soc. Sci. Fish.*, Vol. 43 (1977), pp. 219-227.
9. R. C. Lundstrom and L. D. Racicot: Gas chromatographic determination of dimethylamine and trimethylamine in seafoods. *J. Assoc. Anal. Chem.*, Vol. 66 (1983), pp. 1158-1163.
10. M. Yamata, K. Horomito, and C. Nagoaka: Assessment of green tuna: Determining trimethylamine oxide and its distribution in tuna muscle. *J. Food Sci.*, Vol. 34 (1969), pp. 156-159.
11. N. R. Jones, J. Murray, E. I. Livingston, and C. K. Murray: Rapid estimations of hypoxanthine concentrations as indices of the freshness of chill stored fish. *J. Sci. Food Agric.*, Vol. II (1964), pp. 763-774.
12. J. S. Lee and D. K. Pfeifer: Influences of recovery media and incubation temperatures on the types of microorganisms isolated from seafoods. *J. Milk Food Tech.*, Vol. 37 (1974), pp. 553-556.

13. T. O. Takahashi: Squid meat and its processing. In, Fish as Food, (G. Borgstrom, ed.), Vol. 4 (1965), pp. 339-354.
14. N. Ishikawa and K. Nakamura: on board and on land freshness preservation of squid. Prog. Rep. Squid Fish. Survey World, Vol. 5 (1975), pp. 197-199.
15. P. J. Ke, A. Woyewoda, and M. Fierheller: Handling methods and quality evaluation of fresh Canadian Atlantic squid (*Illex illecebrosus*). Fisheries and Marine Service Technical Report No. 898, (1979), Dept. of Fisheries and Oceans, Halifax, N. S. pp. 7.
16. A. D. Woyewoda and P. J. Ke: Laboratory quality assessment of Canadian Atlantic squid. Fisheries and Marine Service Technical Report No. 902, (1980). Department of Fisheries and Oceans, Halifax, N. S., pp. 26.
17. H. Ohmori, T. Hori, and K. Nakamura: Studies on the preservation of squid in iced sea water. Reito, Vol. 50 (1975), pp. 435-438.
18. J. R. Botta, A. P. Downey, J. T. Lauder, and P. B. Noonan: Preservation of raw whole short-finned squid (*Illex illecebrosus*) during the period from catching to processing: Skin color of raw squid and sensory quality of the subsequently cooked squid. Fisheries and Marine Service Technical Report No. 855, (1979), Department of Fisheries and Oceans, St. John's Nfld., pp. 21.
19. R. J. Learson and V. G. Ampola: Care and maintenance of squid quality. Mar. Fish. Rev., Vol. 39 No. 7 (1977), pp. 15-16.
20. G. D. Stroud: Squid. Torry Research Advisory Note. No. 77, Torry Research Station, Aberdeen, Scotland, pp. 5.
21. B. M. Slabyj and R. M. True: Preprocess holding of squid (*Illex illecebrosus*) and quality of canned mantles. J. Food Prot., Vol. 44 (1981), pp. 109-111.
22. E. Tanikawa, T. Motohiro, H. Ishiko, K. Fujii and K. Yachi: Studies on the complete utilization of squid. XII. On the difference of decomposable velocities of summer and autumn meat. Bull. Fac. Fish. Hokkaido Univ., Vol. 7 (1956), pp. 49-61.
23. L. Stevens: Squid possibilities are best right here in U. S. Commercial Fisheries News, (Oct. 1981), p. 7.
24. E. Tanikawa, M. Akiba and T. Nakamura: Studies on the complete utilization of squid. XI. Studies on the manufacture of surume (dried squid). 4. On the mustiness of squid meat during its drying. Bull. Fac. Fish. Hokkaido Univ., Vol. 4 (1954), pp. 323-336.
25. T. Tokunaga, H. Iida and K. Miwa: The gas chromatographic analysis of amines in fish. Bull. Jap. Soc. Sci. Fish., Vol. 43 (1977), 219-227.
26. D. W. Stanley and H. O. Hultin: Amine and formaldehyde production in North American squid and their relation to quality. J. Inst. Can. Sci. Technol. Aliment., Vol. 17 No. 3 (1984), pp. 157-162.
27. R. J. LeBlanc and T. A. Gill: Ammonia as an objective quality index in squid. Can. Inst. Food Sci. Technol. J., Vol. 17 No. 4 (1984), pp. 195-201.
28. J. J. Shewan, D. M. Gibson and C. K. Murray: The estimation of trimethylamine in fish muscle. In, Fish Inspection and Quality Control (R. Kreuzer, ed.), (1971), Fishing News (Books), London, pp. 183-186.
29. T. Motohiro and E. Tanikawa: Studies on food poisoning of mollusca especially of squid and octopus meat. 1. Chemical changes and freshness tests of squid and octopus meat during deterioration of freshness. Bull. Fac. Fish. Hokkaido Univ., Vol. 3 (1952), pp. 142-153.

30. J. R. Botta, J. R. Lauder and M. A. Jewer: Total volatile basic nitrogen (TVB-N) as an index of quality of fresh fish. 27th Atlantic Fisheries Technological Conference, Portland, Maine. 1982.
31. J. R. Burt, G. D. Stroud and N. R. Jones: Estimation of hypoxanthine concentrations in fish muscle by a rapid visual modification of the enzymatic assay procedure. In, Freezing and Irradiation of Fish. (R. Kreuzer, ed.). Fishing News (Books), London (1969), pp. 367-370.

CARACTERISTIQUES DE L'ENTREPOSAGE DE CALMAR ENTIER GLACE

RESUME : La durée de conservation dans la glace de calmars entiers était estimée à 11-12 jours par évaluation sensorielle. La détérioration de l'aspect et de la saveur était responsable du rejet du produit. La perte de qualité a, elle aussi, été surveillée par des essais objectifs. On a trouvé une très bonne corrélation entre la saveur et la teneur en amoniac, en TMA (triméthylamine), en TVB (bases volatiles totales) et en hypoxanthine, le pH, l'APC (nombre d'aérobies sur plaque) et l'analyse au Torrymètre. Cependant aucune de ces méthodes n'a permis de distinguer un calmar de 2 jours post mortem de celui de 5 jours, les variations étant très faibles. Au début de la détérioration les divers indices physico-chimiques étaient les suivants : $\text{NH}_3\text{-N} = 29 \text{ mg } \%$, $\text{TMA-N} = 13 \text{ mg } \%$, $\text{TVB-N} = 45 \text{ mg } \%$, $\text{H}_x = 2,4 \text{ } \mu\text{M/g}$, $\text{pH} = 6,8$, $\text{APC} = 1,5 \times 10^6$ et lecture moyenne au Torrymètre = 6,8.

DISCUSSION

Z. KARNICKI (FAO) - Do you have experience of holding squid in chilled seawater ?

R.J. LEARSON - Yes; chilled seawater systems are excellent for maintaining squid quality for short periods of up to 3 to 4 days. After 5 or more days, however, the water can become contaminated with bacteria and the squid spoil rapidly. The use of CSW depends on the market. If the squid are to be processed into skinless rings or tubes CSW is fine. CSW squid, however, are recognisable and their acceptability on the Japanese whole squid market is low.

SECTION VII

**AMÉLIORATION ET CONTRÔLE
DE LA DISTRIBUTION TRADITIONNELLE
DES POISSONS RÉFRIGÉRÉS**

***IMPROVING AND MONITORING TRADITIONAL
HANDLING OF CHILLED FISH***

CONDUCTANCE MEASUREMENT AS A METHOD FOR DETERMINATION OF THE BACTERIOLOGICAL AND ORGANOLEPTIC QUALITY OF CHILLED FISH

L. GRAM

Institute of Hygiene and Microbiology
Royal Veterinary and Agricultural University,
Copenhagen (Denmark)

1. INTRODUCTION

Evaluation of the quality of fish and fish products normally includes an estimation of the number of microorganisms by plate counting. Although widely used, some disadvantages of the traditional plate counts are to be recognized. These procedures are laboursome, automation cannot be accomplished and incubation periods of several days are often required. As a consequence, the data obtained cannot be used as a tool in direct processing control. Furthermore estimation of microbial numbers seldom provide information on the freshness and organoleptic quality of the fish.

To overcome some of these disadvantages a number of 'rapid microbiological methods' have been proposed and amongst the most promising in this field are automated conductance and impedance measurements.

Microorganisms growing in a liquid medium produce chemical changes which alter the electrical conductance or impedance. These changes are detectable when the level of microorganisms reach 10^6 - 10^7 CFU/ml /1/. The time required to produce a significant conductance or impedance change, i.e. the detection time, is dependent on the initial concentration of microorganisms and their growth kinetics at the conditions determined by the temperature and growth medium chosen. A detailed description of the theoretical aspects of the conductance measurement has been given by Richards et al. /2/.

Several works have investigated the use of conductance or impedance measurements for the rapid estimation of microbial load in food samples, and high correlations have been demonstrated between detection times and traditional colony counts /1,3,4/.

Since the conductance changes are not only a reflection of the number of microorganisms present but also of their actual metabolic activity, the detection time may correlate to a higher degree with the organoleptic changes during spoilage of fish than the colony count.

The aim of the present study was to evaluate the applicability of conductance measurements for determination of the quality of cod fish. Of special interest in this work was a comparison of detection times with taste panel scores and an investigation of the microbial flora responsible for the conductance change.

2. MATERIALS AND METHODS

Media

Physiological saline containing 0,1% peptone (PS) was used as diluent. Tryptone Glucose Extract (TGE; Difco) was used for psychrotrophic counts. Total plate counts and H_2S -producing organisms were enumerated using a peptone-iron agar (PI): 0,3% beef-extract; 0,3% yeast-extract; 2% peptone; 0,5% NaCl; 0,03% ferric citrate; 0,03% sodium thiosulphate;

pH=7,4. For conductance measurements were used, Brain Heart Infusion Broth (BHI, Difco) and a trimethylamine oxide medium (TMAO) /5/: 0,5% peptone; 0,25% glucose; 0,5% NaCl; 0,1% K_2HPO_4 ; 0,1% $MgSO_4$; 0,1% TMAO; pH=7,2. Disturbance of the conductance curves by settling of fish particles was prevented by adding 0,2% agar to both growth media.

Fish samples / Test procedure

3 Lots each containing 30 freshly caught cod fish were stored at 0°C (iced). 5 Times during a storage period of 14 days samples of 6 fish were withdrawn from each lot. On the days of examination the following analyses were carried out: organoleptic assessments, microbiological counts and conductance measurements.

Organoleptic assessments

Fillets of the 6 fish withdrawn were poached and odour and flavour were evaluated by a panel of 6 judges. The evaluations were made using a scoring system from 10 to 0 points, where scores of 10 to 6 indicated a fresh fish of grade I and scores of 6 to 4 a fish of grade II where slight off-odours/-flavours could be recognized. Scores below 4 points were given when the fish were considered organoleptically rejectable.

Microbiological counts

A 10^{-1} suspension was prepared by homogenising 10 g of belly flaps with 90 ml of PS. Serial 10-fold dilutions were prepared in PS.

Psychrotrophic counts were determined by pour plating 1 ml of appropriated dilution with TGE. Plates were incubated at 7°C for 10 days.

H_2S -producing organisms and total plate counts were determined by pour plating with PI. After solidification, a cover layer of PI was added and plates incubated at 20°C for 3 days. Black colonies were counted as H_2S -producing bacteria.

All plate counts were done in duplicate.

Conductance measurements

In this study, conductance measurements were carried out on the Malthus Microbial Growth Analyser (8-channel) produced by Malthus Instruments Ltd., William Glowes Street, Burslem, Stoke-on-trent. The instrument has been described by Baynes et al. /6/.

Portions of 1 ml of the primary suspensions were pipetted into the glass ampoules. These contained reusable platinum electrodes printed on a ceramic board and had been autoclaved with 9 ml BHI or TMAO. After being adequately mixed the glass ampoules were incubated in a precisely controlled water bath and connected to the control box. The conductance was measured continuously on an Euroscribe flatbed recorder from A. Gallenkamp and Co. Ltd., PO Box 290, Christopher Street, London.

All measurements were done in duplicate.

Investigation of the bacterial flora

Composition of the psychrotrophic flora was determined by isolating colonies from 7°C/10 days-plates containing 20-100 colonies. Isolation of the flora responsible for the change in conductance was done as follows: after measurements had been terminated, serial 10-fold dilutions were prepared from the glass ampoules; 1 ml of appropriate dilutions was, pour plated with TGE and plates incubated at 20°C for 3 days, whereupon colonies were isolated.

The identification procedure included: Gram staining properties, shape, motility, glucose breakdown (oxidation/fermentation), catalase- and cytochrome oxidase reactions. It was determined whether isolates from the conductance glass ampoules could be characterized as 'psychrotrophs' i.e. were able to form colonies at 7°C within 10 days on TGE. All isolates were tested for their ability to reduce TMAO and to produce H_2S when decomposing cysteine.

3. RESULTS

Conductance curves

Both media produced conductance curves characterized by stable, smooth baselines followed by sharp, distinct accelerations which made exact determination of detection times possible. These qualities were most pronounced when using the TMAO medium which can be seen from figure 1., where typical appearance of conductance curves is shown.

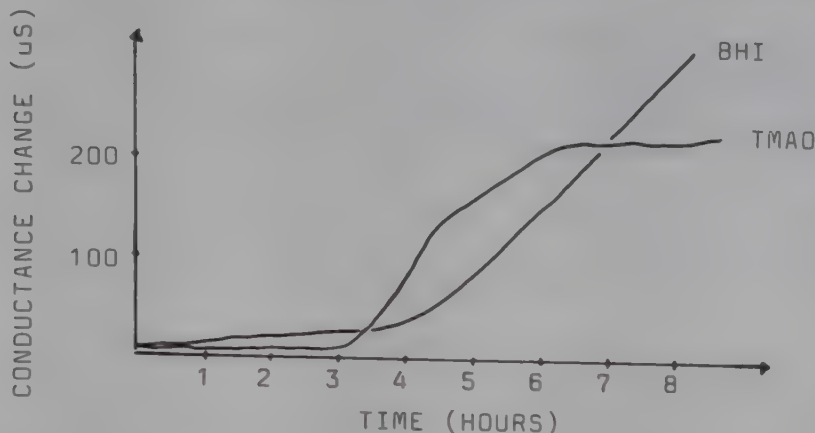


Figure 1. Effect of growth medium (BHI and TMAO) on the shape of conductance curves of 10^{-2} suspensions of cod.

Plate Counts vs. Detection Times

The detection times of 15 samples of cod fish were compared with all colony counts and regression lines, correlation coefficients and standard deviations for all possible combinations are presented in table I. In figure 2 detection times are plotted against psychrotrophic counts and it appears that 10^6 - 10^9 CFU/g were detected within 12-4 hours when conductance changes were measured using BHI as growth medium and within 10-3 hours when TMAO was used.

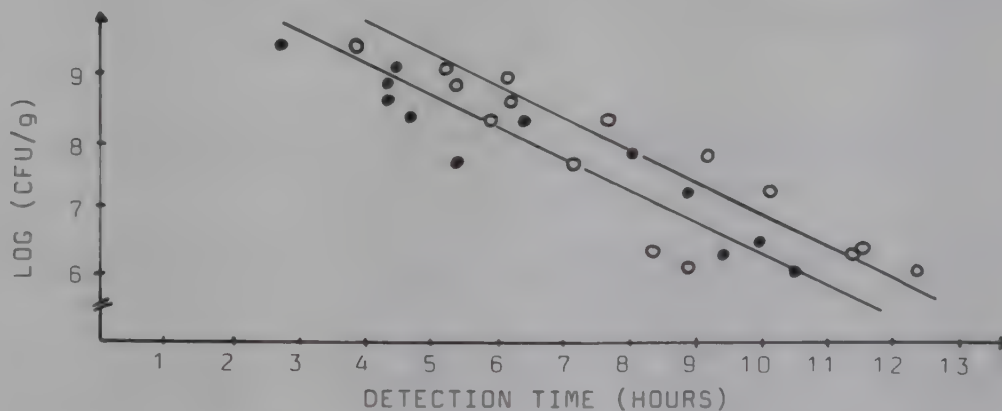


Figure 2. Comparison of initial psychrotrophic counts and detection times assayed in BHI (○) and TMAO (●).

MEDIUM	COLONY COUNT	REGRESSION-EQUATION	R	S _{x,y}
TMAO	7 ⁰ /10d	Y = -0,43 DT + 10,72	-0,95	0,14
BHI	-	Y = -0,43 DT + 11,14	-0,90	0,33
TMAO	20 ⁰ /3d	Y = -0,46 DT + 10,85	-0,95	0,06
BHI	-	Y = -0,45 DT + 11,33	-0,92	0,22
TMAO	H ₂ S/3d	Y = -0,54 DT + 10,72	-0,98	0,06
BHI	-	Y = -0,52 DT + 11,19	-0,95	0,22

Table I. Comparisons of colony counts (=Y) and detection times (=DT) by linear regression analysis. R = correlation coefficient; S_{x,y} = standard deviation.

The detection times obtained when fish samples were assayed in the TMAO medium were approximately 1 hour shorter than when using BHI. This medium also gave the best correlation with plate counts and the least standard deviation along the regression lines.

Quality Score vs. Detection Times

Figure 3 shows the relationship between results of the organoleptic assessments and the detection times determined by conductance measurements of the examined samples.

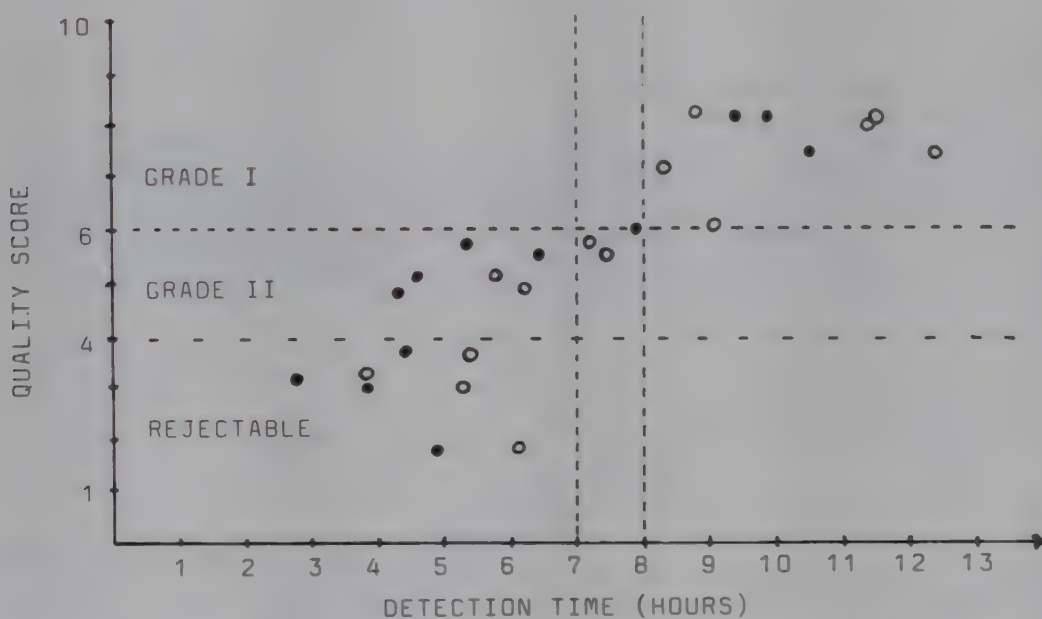


Figure 3. Comparison of organoleptic assessments and detection times determined in BHI (●) and TMAO (○).

The scattergram thus obtained could be used to divide the fish samples into categories of different organoleptic qualities. The results show that selecting a cut-off time of 7 hours (TMAO) or 8 hours (BHI) made it possible to determine whether the fish was of grade I or of poorer quality. A similar differentiation between grade II qualities and rejectable samples was, however, not possible.

Composition of the bacterial flora

A total of 44 psychrotrophic isolates were investigated and results of the identification procedures showed that the microbial flora

was dominated (89%) by Gram negative, non-fermentative rods of the genera Moraxella, Acinetobacter and Pseudomonas/Alteromonas. 9 of these strains (20% of the total number) were able to reduce TMAO and to produce H_2S whereas the remaining 35 strains possessed none of these characters.

27 Strains from TMAO ampoules and 37 from BHI ampoules were investigated and it was found that Gram negative, motile rods able to attack glucose fermentatively constituted the main part of the conductance flora (93 and 78%, respectively). Approximately half of the BHI-strains were Vibrio-like organisms, whereas this group accounted for 3/4 of the TMAO-flora.

31 Of the BHI strains were able to reduce TMAO and of these, 10 strains were also able to produce H_2S . Of the 27 strains isolated from TMAO ampoules 25 were able to utilize TMAO as electron acceptor and only 2 produced H_2S when decomposing cystein.

All of the 64 'conductance isolates' could be classified as psychrotrophs when the ability to form visible colonies at 7°C within 10 days was used as criterion.

4. DISCUSSION

It is assumed that the conductance changes arising from bacterial growth are a result of the accumulation of charged metabolites and preliminary experiments /7/ have indicated that especially transformation of nitrogen containing compounds is the cause of the changes observed.

As pointed out by Gibson et al. /3/, the TMAO-molecule is ideal when seeking a substrate for conductance evaluation of fish and fish products. This N-containing molecule is uncharged and the reduction to TMA, which is highly charged at neutral pH, is required for the spoilage flora which under microaerobic/anaerobic conditions utilize TMAO as electron acceptor.

In this study it was found that conductance changes were detected at an earlier stage when samples were inoculated in the TMAO-substrate as opposed to BHI. This is in agreement with previous experiments /3/ and may partly be caused by the more rapid accumulation of ionized substances i.e. TMA. These observations thus demonstrate the need for development of special conductance media based upon an understanding of the physical factors affecting this parameter.

The microbial flora will be composed of microorganisms with different growth kinetics and at a specific temperature the generation times of the different species will vary considerably. Easter et al. /8/ showed that the generation time of Vibrio ssp. at 20°C is much shorter than of the species dominating the fish flora at 0°C e.g. Alteromonas ssp. Since the TMAO medium strongly favoured Vibrio-like organisms this is also a cause of the more rapid detection times observed in this medium.

Investigation of the different bacterial floras showed that the conductance flora differed from the dominating psychrotrophic flora. A well-known fact was thus demonstrated: the isolation procedures normally followed will only represent groups of microorganisms present in high levels and not minor groups of the microflora. Apparently Vibrio-like organisms are present on the fish and the level of this population closely follows the total concentration of microorganisms since close correlation was found between detection times and colony counts.

Probably, the conditions in the conductance assay determined by the temperature/substrate combination will always favour a very small part of the microbial population. Bülte and Reuter /4/ investigated beef carcasses by means of conductance and impedance measurements and found that samples with unexpected short detection times contained extraordinary high levels of Enterobacteriaceae and vice versa. Although the conductance flora was not investigated, the results/4/ indicate that the conductance changes were primarily caused by members of Enterobacteriaceae.

In these experiments very high correlations were found between detection times and colony counts. Correlation coefficients varied between 0,90 and 0,98 which is of the same magnitude as demonstrated by other groups /1,3,4/. 10^6 CFU/g were detectable within 11-14 hours and it should be emphasized that using more concentrated suspensions than 10^{-2} will probably shorten detection times considerably.

The organoleptic changes observed during the first period of the spoilage of fish are primarily caused by autolytic processes, whereas the later developments are resulting from bacterial activity. It is evident from the data presented here that the conductance detection time can be used to determine whether or not the bacterial spoilage processes have commenced and thus grading the fish as being of 1. or of quality is possible.

In conclusion, the conductance measurements constitute a very rapid method for estimation of bacterial levels in chilled (iced) fish. Conductance changes were detectable within 14 hours (at maximum) and, in comparison, the traditional plate counts require 3-5 days before results are available. Also, the conductance measurements must be considered as a very valuable tool for the fish processor since a rapid, objective assessment of organoleptic quality seems possible.

5. REFERENCES

1. R. FIRSTENBERG-EDEN: Rapid estimation of the number of microorganisms in raw meat by impedance measurement. Food Technol., vol. 37 (1983) pp. 64-70
2. J.C.S. RICHARDS, A.C. JASON, G. HOBBS, D.M. GIBSON, R.H. CHRISTIE: Electronic measurement of bacterial growth. J. Phys. E: Sci. Instrum., vol. 11 (1978) pp. 560-568
3. D.M. GIBSON, I.D. OGDEN, G.HOBBS: Estimation of the bacteriological quality of fish by automated conductance measurements. Int. J. Food Microbiol., vol. 1 (1984) pp. 127-134
4. M.BÜLTE, G. REUTER: Impedance-measurements as a rapid method for the determination of the microbial contamination of meat surfaces proved by two different instruments. Int. J. Food Microbiol., vol. 1 (1984) pp. 113-126
5. A.J. WOOD, E.A. BAIRD: Reduction of trimethylamine oxide by bacteria 1: The Enterobacteriaceae. J. Fish. Res. Bd. Can., vol. 6 (1943) pp. 194-201
6. N.C. BAYNES, J. COMRIE, J.H.PRAIN: Detection of bacterial growth by the Malthus conductance meter. Med. Lab. Sci., vol. 40 (1983) pp. 149-158
7. A.E. CLARK, D.J. MILLBANK: Factors affecting conductance signal produced by growing bacteria. Poster presented at the 4th Int. Symp. on Rapid Methods and Automation in Microbiology and Immunology, Berlin (1984).
8. M.C. EASTER, D.M. GIBSON, F.B. WARD: A conductance method for the assay of bacterial trimethylamine oxide reduction. J. Appl. Bacteriol., vol. 52 (1982) pp. 357-365

MESURE DE LA CONDUCTANCE POUR DETERMINER LA QUALITE BACTERIOLOGIQUE ET
ORGANOLEPTIQUE DU POISSON REFRIGERE

RESUME: La qualité organoleptique et bactériologique de cabillaud réfrigéré a été évaluée par des mesures de la conductance et, d'après les temps de détection, on a pu classer les poissons en catégories de différentes qualités organoleptiques. Les coefficients de corrélation entre les temps de détection et le nombre des colonies étaient de l'ordre de 0,90 à 0,98. Les temps de détection variaient de 2,7 à 11,4h, les dénombrements sur plaque de 10^5 à 10^9 UFC/g.

Les variations de la conductance ont été surtout provoquées par des bactéries susceptibles d'attaquer le glucose par fermentation et de réduire l'oxyde de triméthylamine (souches semblables à des vibrions), tandis que la flore dominante trouvée sur le poisson était incapable d'utiliser le glucose et variait du point de vue de la production de triméthylamine (souches de Moraxella, d'Acinetobacter et de Pseudomonas).

DISCUSSION

D. VICKERS (U.K.) - A real need in industry is a rapid method for the assessment of bulk purchases of frozen fillets by non expert staff. The work described is with fresh fish but is it also valid for frozen fillets ?

L. GRAM - My immediate answer is 'Yes' but I have not done work on frozen fish and have no knowledge of the method being used for this purpose. It may be that the detection times will be longer than with unfrozen fish due to sublethal damage of the bacteria. This means that the "cut off times" will have to be established by storage trials. It is possible, however, there may be no agreement between organoleptic quality and the conductance detection time when examining frozen fish since the decline in organoleptic quality in frozen fish is not the same extent as in fresh fish as determined by bacteriological developments.

HANDLING AND STORAGE OF
COD ON BOARD INSHORE BOATS

M. HANUSARDOTTIR

J. ZACHARIASEN

L. MOHR

Hygiene Institute, Fisheries & Veteri-
nary Dept., Tórshavn, Faroe Islands

1. INTRODUCTION

It is well known that one of the most important prerequisites for getting an optimal quality of fish is rapid chilling after catching and storage at a temperature of 0°C.

Further, it is of importance that the fish is washed properly and is not being molested, in particular not during the rigor mortis phase (19,21/).

It is, however, generally accepted that the fish from our inshore boats should be of the very best quality in spite of the fact that this fish is not iced on board. This means that the fish is stored for 16-24 hours before chilling. Also the fish is often in rigor, when handled during landing and during a possible washing process.

It is therefore comprehensible that some of the factories claim that the quality of fish from inshore boats is very heterogeneous.

Particularly during the summer the fish has a poor keeping quality and the fish meat has a bad consistency with gaping tendency of the fillets.

Of course, biological variations of the fish may contribute to the changing quality (/20/). However, the above mentioned unfavourable treatment of the fish must also be of importance.

Because of the relatively bad profitability in dealing with inshore fishing, the crew has been reduced to three persons and in some cases even to two persons. At the same time, the fishing operation has been made more effective, which means less time to handle each of the fish in the right way.

There is no chilling of the fish on board and the fish is often badly washed or not washed at all after exvisceration.

Based on preliminary results presented at a fish technology conference in Iceland in September 1984 (/5/) the aim of the present study was to make further investigation on improving the quality and rationalize the handling on board by using iced sea water for bleeding cod and rapid chilling on board, while gutting and washing should be carried out ashore.

This was compared with traditionally handled fish as well as fish, which was bled and gutted before storing in iced sea water.

2. EXPERIMENTAL

Cod were caught with long line about 10 miles off the western side of Faroe Island, on the night between 5 and 6 March 1985. About 100 fish were randomly treated as follows:

- 1) traditional handling, which means bled and gutted in one operation in containers without ice (TRAD),
- 2) bled and gutted in one operation and immediately placed into insulated containers with iced sea water (CSW/G) and
- 3) bled and immediately placed ungutted into insulated containers with iced sea water (CSW/UNG).

The bleeding was carried out by a ventral incision severing the ventral blood vessels. About 10 hours after landing (the trip lasted about 16 hours) the traditionally handled fish was iced. At that time the temperature in the fish meat was 6-7°C. The temperature in the fish in iced sea water (CSW/G and CSW/U) remained on

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

-0.5°C - -1.0°C during storage.

The containers used were 250 l's insulated METAS containers. About 40 vol % of ice was filled into the containers on shore. The sea water was let to the containers immediately before hauling the long line.

At intervals during storage, 5-6 fish from each of the treatments were removed for examination. Further one sample of sea water from each of the CSW treatments were examined.

3. ANALYSIS

3.1. Chemical tests. Analyses carried out were total volatile nitrogen (TVN) according to Jensen (/10/) and Hanusardóttir et al (/6/). The salt content was analysed according to AOAC Methods (/1/).

3.2. Microbiological tests. Total plate count (incubated at 20°C) was estimated by plating serial dilutions of fish samples on iron-plate agar (/2/). H₂S producing organisms were counted on iron-plate agar as black colonies. It has been shown that these are species of spoilage bacteria (*Pseudomonas putrefaciens* or *Alteromonas putrefaciens*) (/12,18/). These tests were carried out in duplicate where each sample was made up by meat from two or three fish.

3.3. Organoleptic properties. The quality of the fish from each of the treatments was followed by organoleptic testing of the raw whole and gutted fish as well as the raw fillets.

Further samples were cooked in plastic bags for 15 minutes and tested by a panel of 5-6 members.

The raw fish quality was evaluated by discussion in connection with the testing, whereas the quality of the cooked fish was evaluated by giving numerical scores for odour, flavour and texture on a scale where 10 represents the ideal fresh product and 0 the lowest quality, while 4 means the limit of acceptable quality.

4. RESULTS

4.1. Chemical. The salt content in the fish treated in traditional way was 0.1-0.2%, whereas the salt values for the fish in sea water were increasing from about 0.1 to about 0.5% during a storage period of 12-14 days. In the same period the salt content in the iced sea water decreased from about 2.1 to 1.4%.

The TVN development during the first 9-10 days was practically the same for the different treated fish (Fig. 1).

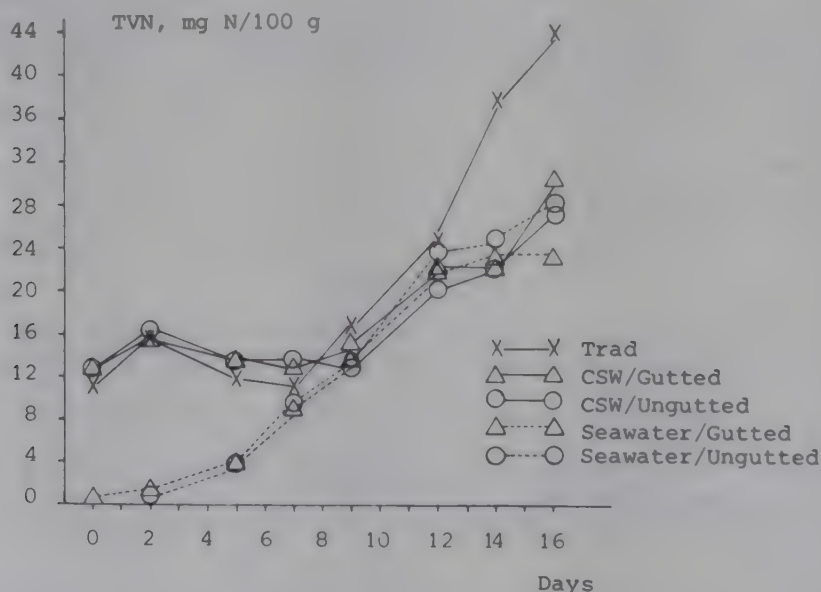


Fig. 1 - TVN content of different treated cod and sea water in which different treated cod was stored.

However after 9-10 days the TVN in the traditional handled fish increased to 44 mg TVN.N/100 g after 16 days, whereas the TVN in the fish in iced sea water remained at about 22 mg N/100 g until the 14'th day of storage and increased to 28-30 mg N/100 g after 16 days. In the same period of time TVN in the sea water increased from 1 to 23 - 17 mg N/100 g, which is about the same level as found in the fish in iced sea water after 16 days. The relatively early increase in TVN in the sea water is probably due to leach out of TMAO into the sea water, which is in accordance with Hanusardóttir and Joensen (/5/) and Landfald (/14/).

4.2. Microbiological. Total plate count at 20°C (psychrophilic flora) and the growth of H₂S producing organisms in the variably treated fish are shown in Fig. 2 and Fig. 3 respectively. In both case the level of the bacteria was almost the same for the different treated fish up to 7-9 days. After 7-9 days the total plate count and the H₂S-producing organisms in the traditionally iced fish were clearly higher. The bacteria content in the sea water (Fig. 4) was generally on a higher level than the corresponding fish stored in sea water. However this difference was eliminated during the storage.

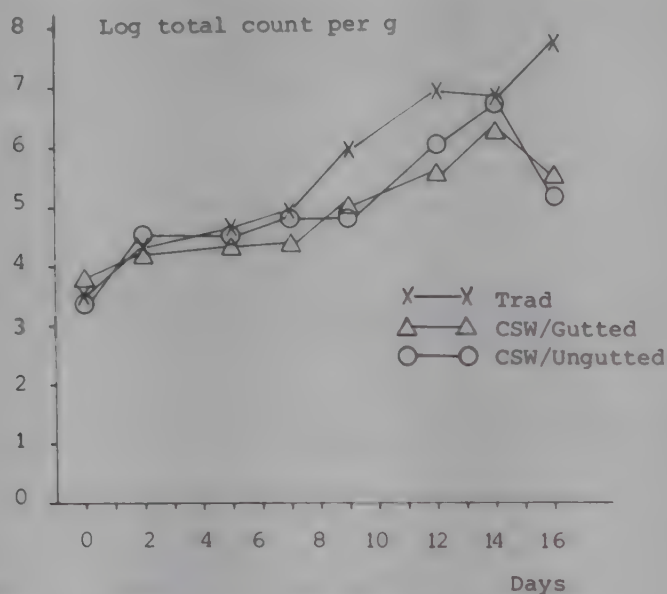


Fig. 2 - Log total count per g in different treated cod.

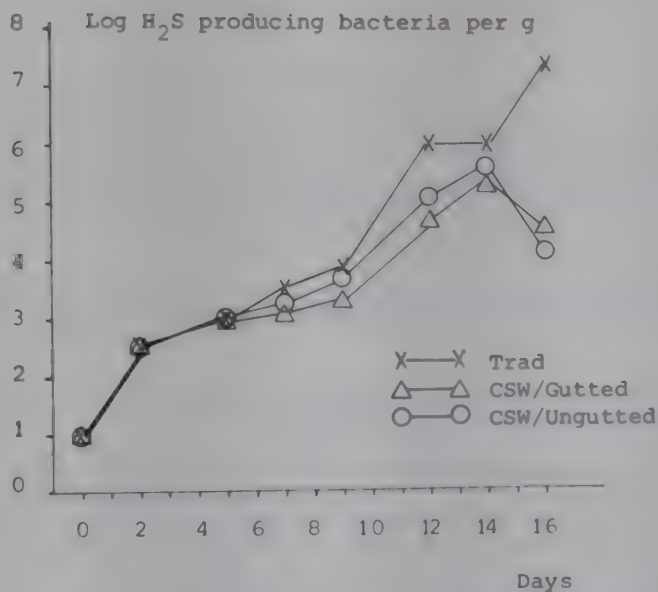


Fig. 3 - Log H₂S-producing organisms per g in different treated cod.

In all fish and, in particular, in the sea water there was increasing pre-dominance of H_2S -producing organisms during the storage (Fig. 5). This tendency was indeed evident in the sea water, whereas the effect was much smaller in the corresponding fish stored in the sea water.

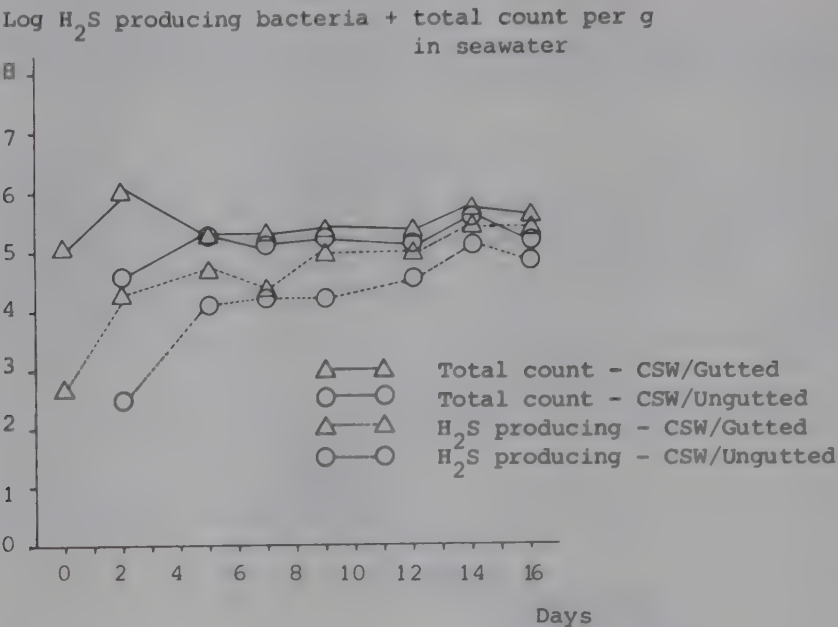


Fig. 4 - Log total count and log H_2S producing organisms per g in iced sea water, in which different treated cod was stored.

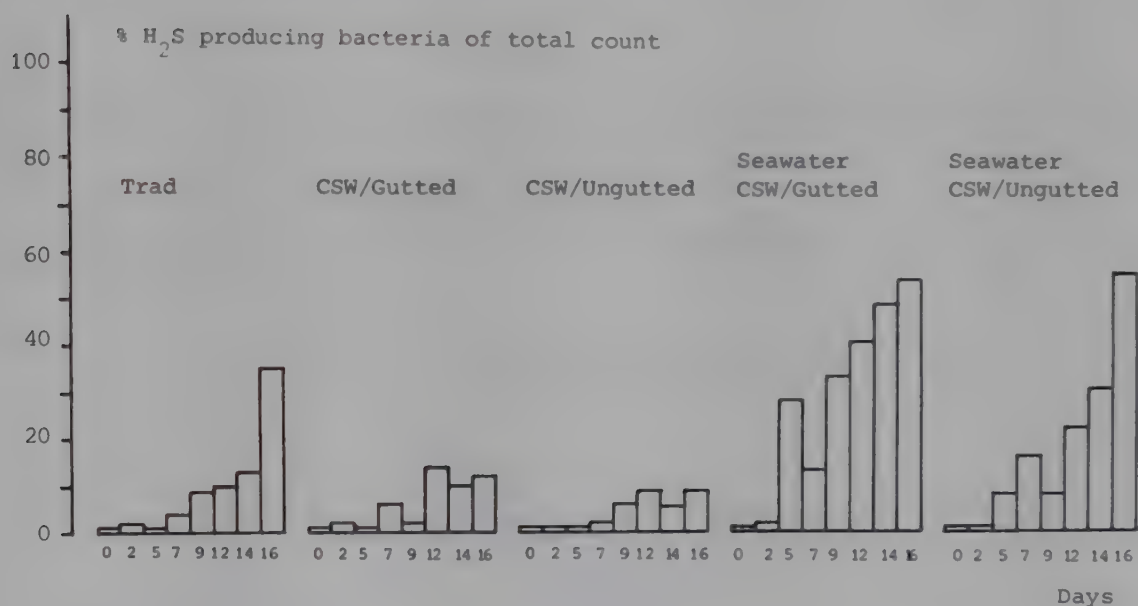


Fig. 5 - H_2S -producing organisms as per cent (%) of total count (20°C).

4.3. Organoleptic. During the storage of 12-14 days, there was no significant difference between the quality score for cooked fillets.

However, the cod in iced sea water had a generally better and more even appearance of the skin and a firmer texture compared to the traditionally handled fish. Further a spoilage smell in the traditionally handled fish showed up already after 5 days whereas the bled and bled/gutted fish in iced sea water had a neutral smell in the belly region until 7 and 9 days respectively.

After 5-7 days a greenish colour from the gall bladder began to show up in the left belly flap of the bled ungutted cod in iced sea water.

5. DISCUSSION

Storage and transport of ungutted small fish species as mackerel, herring and anchovy in iced or refrigerated sea water have been studied and known for a long time (/2,7,9,11,16,17/).

One of the purposes of this study was to make further investigations (/5/) of the possibility of using iced sea water for cod either bled or bled and gutted before placing in sea water.

The results have shown that the use of iced sea water gives a better organoleptic property of the cod when landed, and during the first week of storage compared to the traditionally handled cod. However, this difference did not express itself either through the growth of bacteria or according to the TVN development. On the other hand the traditionally handled cod clearly had the highest level of both TVN and bacteria in the last part of the storage period, in spite of the fact, that the cod in iced sea water at this stage had a much higher degree of a putrid flavour.

Theseresults are not in accordance with Danish experiments (/8/), where discolouring and bad smelling already showed up after 2 days' storage of unbled, ungutted cod. However, good agreement is found with similar Norwegian experiments (/15/) as well as some Faroese results for bled whole saithe (/3/).

It has to be stressed that the fish used in this study had almost empty stomachs, and therefore the activity of the entrail enzymes was relatively low.

In previous studies in September 1983, when the fish should be in a better condition we found that bled, ungutted fish (mainly haddock and smaller amount of cod) in chilled sea water were of good quality until 5-6 days after catch (/4/).

It is obvious that rapid chilling and transport in containers must be the method by which the fish is best protected against any damage, providing the iced supply is sufficient and the fish is not kept in the sea water for too long. In fact, there should be no reason for storing fish from the inshore boats for more than 2-4 days before processing. The question concerning gutting on board or ashore (in the factory) must be a matter between the fishermen and the factory (buyer).

The fish in iced sea water remained at a temperature of about -1°C . The consistency of the fish was therefore very firm as in an artificial rigor stage and needed therefore to be warmed about $2-4^{\circ}\text{C}$ before processing.

The TVN value in the sea water increased from zero to nearly the level of the corresponding fish. Further, a very high selective effect of H_2S producing organisms was found in the sea water compared to the fish meat. This phenomenon is probably caused by leaching out TMAO to the anaerobic sea water (/5,14/) together with the theory that the H_2S producing organism *Alteromonas putrefaciens* probably has the greatest growth potential in anaerobic marine environment (/15,22/).

6. CONCLUSION

Fillets from both bled ungutted and bled and gutted cod caught in March and stored in iced sea water were of very good quality during the first 9 days of storage at a temperature of $-0.5 - -1.0^{\circ}\text{C}$.

The cod in iced sea water had a generally better and more even appearance of the skin and a firm texture compared to the traditionally handled fish.

After nearly a week of storage of the ungutted cod a greenish colour from the gall bladder began to show up in the left belly flap followed by an increasing bad smell in the stomach region.

The salt content in the fish stored in iced sea water, which was about 0.5% after a period of 12-14 days, had no influence on the organoleptic properties.

The cod stored in iced sea water had a less pronounced net growth of bacteria and a lower TVN development than the traditionally iced cod. There was a much higher degree of selection of H_2S -producing bacteria in the sea water compared to the fish meat. This is probably the reason for the marked putrid flavour of the fish in iced sea water at the end of the storage period.

Chilling and storing of gutted and/or bled cod seems to be a realistic way of improving and optimizing the handling on inshore boats provided that the fish is not stored in the sea water for more than 5-6 days.

REFERENCES

1. AOAC Methods. 11th Ed. 296. Ass. of Off. Analyt. Chem., Washington (1970).
2. Hansen, P. and Larsen, E. Nedkøling og før. ... Fisk.Forsøgslab., Lyngby (1981).
3. Hanusardóttir, M. Hagreiðing-Snarkøling. Heilsufr. Starvsst., Tórshavn (1982).
4. Hanusardóttir, M. and Joensen, O. (1983). Ilandbringn. .. H.S., Tórshavn (1983).
5. Hanusardóttir, M. and Joensen, O. Fish Handling on Board Inshore Boats. In Press. Symp. from Fish Techn. Conference, Iceland (1984).
6. Hanusardóttir, M., Erenbjerg, A. and Poulsen, A. En sammenligning mellem to metoder ... (TVN). In Press, Dansk Vet. Tidsskrift (1985).
7. Hulme, S.E. and Baker, D.W. Chilled Seawater System for Bulkholding Sea Herring. MFR paper 1238. Marine Fisheries Review, 39 (3) (1977) pp 4-9.
8. Huss, H.H. and Asenjo, I. Storage Life of Guttet and Unguttet White Fish. Annual Report pp 32-47. Technological Laboratory, Ministry of Fisheries, Denmark (1976).
9. Jensen, J. Bratkøling af ... Abstract from: Dansk Fiskeritidende, No 32 (1981).
10. Jensen, E.P. Hurtig TVN-analyse ved vanddampdest., Biotekn. Inst., Kolding (1981).
11. Jensen, J.G. and Hansen, P. Bulk handling and chilling of large catches of small fish. Part II: Purse seine catches. Infofish Marketing Digest, 1 (1983) pp 34-38.
12. Jensen, M. Hanusardóttir and Schultz, E. Jernagar's anvendelse til friskhedsbestemmelse af fersk fisk. Dansk Vet. Tidsskr., 63 (8) (1980) pp 314-318.
13. Jensen, M. Hanusardóttir, Petersen, A. and Røge, E. Hartvig. Storage of Chilled Cod ... Adv. in Fish Sc. and Techn., I.I.Connel ed, p. 294, Fishing News Books Ltd., Surrey, England (1980).
14. Landfald, B. Lagring af torsk ... Fiskeritekn. Forsk.inst., Norway (1977).
15. Landfald, B. and Adolfsen, H. "Is-sjøvannscontainere i kystfiskeflåten..." Rapport nr. 663.5-2-1, Fiskeritekn. Forskningsinstitut, Tromsø, (1980).
16. Lauterback, N. (1982). Coding the catch. Small boat refrigeration. Fishing News International (5) (1982) pp 69.
17. Lemon, D. and Regier, L.W. Holding of Atlantic Mackerel (*Scomber scombrus*) in Refrigerated Sea Water. J. Fish. Res. Bd. Can. 34, (1977) pp 439-443.
18. Levin, R.E. Detection and Incidence of specific species of spoilage bacteria on fish. I. Methodology. Appl. Microbiol. 16, (1968), pp 1734-1937.
19. Love, R.M. Gaping of Fillets, Torry Adv. Note, No 61. Torry Res. Station, Aberdeen, Scotland (1973).
20. Love, R.M. Biological factors affecting process. and utilizations. In "Advances in Fish Sc. and Technology", pp 130. J.J. Connell (ed). Fish News, London (1980).
21. Stroud, G.D. Rigor in fish. Torry Advisory Note No 36. Torry Research Station, Aberdeen, Scotland (1969).
22. Strøm, A. Personlige meddelelser. Institut for Fiskerifag, Universitetet i Tromsø, Norway (1985).

MANIPULATION ET ENTREPOSAGE DU CABILLAUD A BORD DE NAVIRES COTIERS

RESUME: Les opérations de pêche, à bord des navires côtiers, étant devenues plus efficaces, le temps de manipulation du poisson a diminué.

Par conséquent, le poisson est souvent mal lavé ou n'est pas lavé après éviscération et il n'y a pas de réfrigération du poisson à bord. De plus, le poisson est le plus souvent à l'état de rigor lorsqu'il est débarqué.

Cette étude a eu pour but de poursuivre les recherches sur les moyens d'améliorer et de rationaliser la manipulation à bord en plaçant le poisson dans de l'eau de mer glacée immédiatement après saignée ou après saignée et éviscération.

On a trouvé que les filets de cabillaud saigné, non éviscéré et ceux de cabillaud saigné et éviscéré, pêché en mars et conservés dans de l'eau de mer glacée étaient de très bonne qualité au cours des neuf premiers jours. Cependant, au bout d'une semaine d'entreposage de cabillaud non éviscéré une couleur verdâtre provenant de la vésicule biliaire apparaissait sur le flanc gauche et elle était accompagnée d'une mauvaise odeur croissante dans la région stomacale.

VARIATIONS IN POTENTIAL SHELF LIFE RESULTING FROM BOXING AT SEA PRACTICE

I. McDONALD and J. GRAHAM
Torry Research Station, Aberdeen, (United Kingdom)

1. INTRODUCTION

The potential shelf life of prepacked chilled fish will depend on the treatment of the fish from the time of catching until they are fully prepared for retail.

The time delay between catching and packaging for marketing is obviously important and this must be taken into account when specifying potential shelf life. The treatment to which the fish are subjected during this period, however, can be variable and, with poor handling and storage practice, a number of days of potential shelf life can be lost.

This paper summarises some recent work relating to current UK boxing at sea practice and quantifies, in terms of loss of potential shelf life, the effect of overfilling boxes used for stowing fish on fishing vessels.

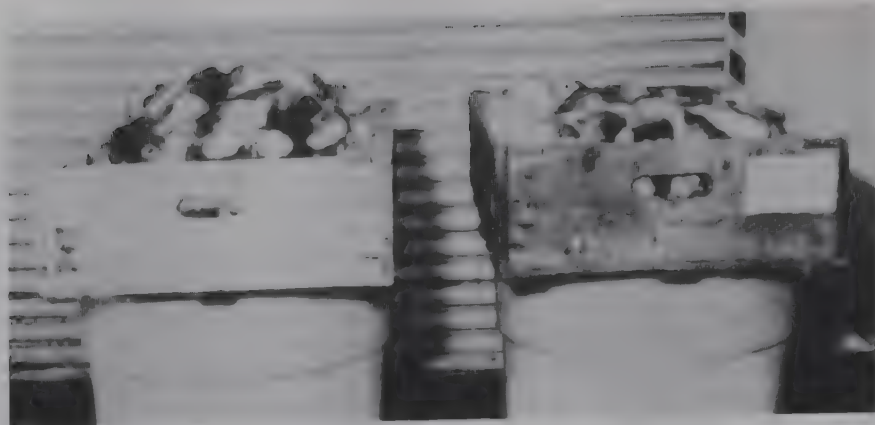
2. STOWAGE OF FISH IN BOXES

It is difficult to identify when boxing at sea was first introduced since the use of containers for fish is an inherent part of fishing. The need for adequate icing and the avoidance of crushing the fish when boxes are stacked was described for a boxing at sea experiment carried out for the Aberdeen Vessel Owners Association in 1928 /1/. The same reference also notes that boxing practice at the French port of Boulogne required the fish to be weighed so that boxes were not overfilled and a guarantee of fish weight could be given to the buyers.

The use of boxes for the storage of fish on fishing vessels is likely to have been a progressive development from the bulk stowage of the fish in the hold in order to improve both fish handling and quality. Boxed fish are contained in relatively shallow containers, therefore, there is no crushing effect. The boxes can be handled without disturbing the fish and this makes it possible to contain the fish in ice from the time of catching until they are ready for processing onshore. Boxing also allows for effective sorting of the fish at sea and on the market floor into grades according to species, size and time caught.

Boxing is, therefore, an effective method of stowing fish but only if it is properly applied. Overfilling of boxes, for instance, can result in some of the benefits being lost and this practice now prevails at major fishing ports in NE Scotland.

Fish boxes are intended to hold a specific weight of fish but, since this is usually gauged by volume, the quantity of fish is variable with a general tendency to overfill the boxes to ensure the correct weight. Overfilling means that there is insufficient space for an adequate quantity of ice to be used to achieve both effective cooling and the maintenances of chill temperatures during subsequent storage and handling. Overfilling also results in a loss in weight during storage due to crushing of the fish and a reduced fillet yield when the fish are processed.



Two boxes of iced fish each containing the quantity of fish frequently found in commercial practice. The box on the left shows the resulting gross degree of overfilling; that on the right shows the crushing and compacting effect of stacking boxes on top of one another.

3. BOX SPECIFICATION

The box used was a standard wooden fish market trunk box commonly used in NE Scotland which is now the major area for UK fish landings. The internal dimensions of the box are 775 mm x 457 mm x 165 mm deep giving a volume of 58.4 litres which is ideally suited to contain 33 kg of fish with ice added to give a fish to ice ratio of up to 3 to 1. Proposals have been made to specify this box for holding 40 kg of fish and this weight has therefore been used in some of the tests.

Boxes have been known to contain up to 60 kg or more of fish but weights of 45 and 50 kg are used in the tests to represent typical commercial overfilling practice /2/.

4. EXPERIMENTAL PROCEDURE

The work reported is based on 2 distinct series of tests, one of which involved 2 trips on a fishing vessel. The experimental procedure was much the same in all tests and the following main conditions were applied:

1. The fish, preferably medium sized haddock, were gutted and washed then allowed to drain for a short period before being weighed then stowed in ice in the boxes as soon as possible after catching.
2. The boxes were stacked 6 or 7 high. This was the maximum possible but on commercial fishing vessels where the headroom is available stacks of up to 8 boxes are used.
3. Commercial icing practice involved placing a layer of ice on the bottom of the box adding the fish then adding more ice on top. A small quantity of ice is sometimes added at an intermediate stage of filling but this was not done in the tests. A sheet of grease-proof paper may also be placed between the fish and the top ice in order to reduce marking of the fish and allow the fish and ice to be readily separated for inspection.
4. Ideal icing practice used in the tests consisted of placing a layer of ice on the bottom of the box adding fish and ice intimately mixed then finishing off with a good layer of ice on top to give an overall fish to ice ratio of 2.5 to 1.

The above conditions were adhered to except when a stack of boxes could not be completed from 2 successive hauls when other species, such as whiting, of similar size were used to fill boxes not required for quality or other assessments.

In the first series of tests comparisons were made between ideally iced boxes /3/ containing 33 kg of fish and 13 kg of ice and commercially iced boxes containing 45 kg of fish. The boxes were stacked 6 high and samples were taken from individual stacks for quality assessment after 3, 7 and 10 days.

In the second series of tests a direct comparison was made between boxes containing 40 kg of fish and boxes containing 50 kg. In both cases commercial icing practice was used and the boxes were stowed 7 boxes high. Fish were removed after 6, 7 and 8 days storage for assessment.

The fish were assessed for raw appearance and cooked flavour by a taste panel using a 10 point scale /4/. The cooked flavour score is considered to be more important and a score of 7 at the processing factory reception is usually aimed for and a score of 6 is about the end of 'good quality' shelf life /5/. A score of 7 should be obtained after about 7 to 8 days in ice if the fish are well cared for.

5. RESULTS

The taste panel scores for raw appearance and cooked flavour for the first series of tests are given in Tables 1 and 2. The scores are average scores for a number of samples removed from boxes located at different levels in each stack.

TABLE I

Series 1 - Raw Appearance Score

Days in Ice	Ideal Icing	Commercial Overfilled
3	8.3	8.4
7	6.8	6.5
10	6.3	6.1

TABLE II

Series 1 - Cooked Flavour Score

Days in Ice	Ideal Icing	Commercial Overfilled
3	8.3	8.7
7	7.0	6.7
10	6.6	5.5

Tables 3 and 4 give the taste panel scores for the second series of tests. Samples for assessment were taken from boxes in the position indicated.

TABLE III

Test Series 2 - Raw Appearance Score

Days in Ice	50 kg box				40 kg box			
	B	M	T	Mean	B	M	T	Mean
6	6.7	7.1	7.3	7.3	6.8	6.6	6.8	6.8
7	5.7	6.1	5.9	5.9	5.9	6.6	7.2	6.6
8	6.2	5.9	6.7	6.3	6.2	6.7	6.5	6.5

TABLE IV

Test Series 2 - Cooked Flavour Score

Days in Ice	50 kg box				40 kg box			
	B	M	T	Mean	B	M	T	Mean
6	6.7	6.7	7.1	6.8	7.4	7.1	7.2	7.2
7	6.7	6.6	6.6	6.6	7.0	7.1	7.1	7.1
8	6.5	6.2	6.9	6.5	6.3	7.0	6.4	6.6

B - Bottom Box
M - Middle Box
T - Top Box

6. DISCUSSION

Although 90% of all UK caught fish is landed within 6 days of capture storage times of up to 10 days are possible if distribution time onshore is taken into account.

Fish in the commercially iced and overfilled boxes in the first series of tests lost 3.2 marks in cooked flavour after 10 days storage whereas ideally iced fish lost only 1.7 marks. The difference is equivalent to about 4½ days storage in ice /6/ and, allowing for the variance in taste panel markings and fish sampling, it would appear that 2 to 3 days of potential shelf life was lost as a direct result of overfilling.

In the second series of tests the difference in quality between the 40 kg and 50 kg boxes was not so pronounced and some of the differences in marking are within the expected variance. However, the 40 kg boxes consistently gave better results and a number of direct comparisons show that after 6 to 8 days storage there was a possible difference of 1 day in the potential shelf life. In the second series of tests an attempt was also made to determine the variation in quality throughout a stack of boxes and the results show that an overall trend for lower quality to be equated with a lower position in the stack applied to both the 40 and 50 kg boxes.

Undericing is inevitable when boxes are overfilled since there is no longer space available for sufficient ice to be used other than for storage periods of no more than a few days. This has been confirmed on a number of occasions and the results from earlier work /7/ show that towards the end of a trip fish temperatures

begin to rise. More recent work, not yet complete, indicates that this temperature rise is more pronounced on vessels which do not have additional fishroom cooling.

Overfilling results in the fish being compacted when the boxes are stacked and this effect combined with the commercial practice of icing on top and bottom only, results in longer cooling times. Compacting of the fish reduces the flow of ice melt-water through the box and, therefore, only the fish in direct contact with ice are cooled quickly. Ideally iced fish at the centre of a box took between 2 and 4 hours to reach 1°C and about 8 hours to reach 0°C whereas fish in commercially iced boxes took 12 and 20 hours respectively. In boxes which were overfilled and a paper overlay was used, the cooling times to 1 and 0°C extended to 16 and 40 hours respectively. In each case temperatures were measured throughout the stack and the above figures are typical values for each condition.

The differences in the initial cooling time due to the method of loading the boxes would account for much of the variation in potential shelf life /8/. It would, therefore, seem the temperature difference rather than pressure resulting from overfilling is responsible for the loss in fish quality.

Overfilling boxes also results in physical damage to the fish overhanging the edge of boxes when they are stacked and pressure on the lower fish results in a higher weight loss during the storage period. In the second series of tests weights were checked and the results show that an additional 15 to 17 kg of saleable fillet weight is lost for every 1000 kg of fish stowed at sea as a direct result of overfilling.

Other tests have confirmed the detrimental effects of overfilling boxes and also shown that the likely variations in quality are more complex /9/. Fish taken from overfilled boxes after landing and transporting to a distant market had a potential shelf life that differed by as much as 2 to 3 days depending on the location of the fish in a single box. The better quality fish were located near the top of the box with the poorer quality fish near the bottom. Based on the observations made in the present series of tests this variation is likely to be the result of uneven cooling. However, in the publication referred to, the presence of stagnant pools of bacteria laden water within the box is quoted as the reason for the quality difference.

7. CONCLUSIONS

The work described revealed that there are a wide range of conditions applicable to current UK boxing at sea practice which may result in significant variations in the subsequent potential shelf life of chilled products.

Investigations are still continuing to cover a wider range of conditions but the present work indicates that there is a problem in defining shelf life, since it will be difficult for the processor to detect and separate fish of a standard quality.

The results show that improved boxing practice would not only extend the potential shelf life but also ensure more uniform fish quality.

The tests also indicate that redefining the box to hold 40 kg of fish and applying a rigid checking system to ensure that the weight is not exceeded, will result in an improvement in fish quality. The quality of the fish, however, still falls short of what can be achieved with ideal icing practice.

8. ACKNOWLEDGEMENTS

G. P. Hill, W. A. Johnston and J. H. Kelman assisted in the work at sea and P. Howgate and Anne Craig organised and carried out the sensory assessments.

REFERENCES

1. A. LUMLEY, J. J. PIQUE, G. A. REAY: The Handling and Stowage of White Fish at Sea: D.S.I.R. Food Investigation Special Report No. 37; 1929; HMSO, London.
2. Torry Research Station: Survey of UK Boxing Practice: 1983; (unpublished).
3. Fish Handling and Processing: 2nd Edition, 1983; HMSO, Edinburgh.

4. J. M. SHEWAN, R. G. MACINTOSH, C. G. TUCKER. A. S. C. EHRENBURG: The Development of a Numerical Scoring System for the Sensory Assessment of White Fish Stored in Ice: J. Sci. Fd Agric.; 1953; No. 6, pp. 283-298.
5. C. K. MURRAY, D. M. GIBSON, J. M. SHEWAN: Quality Control Aspects of Prepacked Fresh and Smoked Fish: Fish Inspection and Quality Control; Ed. R. Kreuzer; pp. 66-70; Fishing News (Books); 1971.
6. J. R. BURT, D. M. GIBSON, A. C. JASON, H. R. SANDERS: Comparison of Methods of Freshness Assessment of Wet Fish, 1 - Sensory Assessment of Boxed Experimental Fish; J. Fd Technol, 10, pp. 645-655, 1975.
7. Torry Research Station Annual Report; 1979; p. 4.
8. J. OLLEY, D. A. RATKOWSKY: Temperature Function Integration and its Importance in the Storage and Distribution of Fresh Foods Above the Freezing Point; Fd Tech. in Aust.; 25; (2); pp. 66-73; 1973.
9. P. WILSON: An Investigation of the Effects of Overfilling Boxes at Sea Fish on Quality and Yield; S.F.I.A. Tech. Report No. 229; 1982.

VARIATIONS DU POTENTIEL D'APTITUDE A L'ENTREPOSAGE DU AU PROCEDE DE MISE EN CAISSE A BORD DU BATEAU.

RESUME : Il est indispensable, pour définir l'aptitude à l'entreposage de poissons réfrigérés, de connaître leur qualité et de savoir si cette qualité est uniforme. Il est aussi souhaitable d'accroître l'aptitude à l'entreposage, ne serait-ce que d'un jour, pour améliorer la logistique et la rentabilité aussi bien que la qualité générale du produit. Des recherches sur les techniques de mise en caisse des poissons, à bord du bateau, couramment employées au Royaume-Uni, montrent que la perte de potentiel de conservation du poisson et la variabilité de sa qualité sont directement dépendant du mode de remplissage des caisses. Après un entreposage de 7 jours, l'aptitude à l'entreposage peut être réduit jusqu'à 3 jours et d'importantes variations peuvent exister dans un même lot de poissons et même dans la même caisse. Les auteurs définissent le procédé de glaçage idéal, estiment quel est le profit de son adoption à la place du procédé usuel et proposent une méthode de glaçage.

EVALUATION OF AUTOMATED
TIME-TEMPERATURE MONITORING SYSTEM
IN MEASURING THE FRESHNESS OF CHILLED FISH

B. L. TINKER
National Marine Fisheries Service, Gloucester, Massachusetts
J. W. SLAVIN
Joseph W. Slavin and Associates, Annandale, Virginia
R. J. LEARSON
National Marine Fisheries Service, Gloucester, Massachusetts
V. G. AMPOLA
National Marine Fisheries Service, Gloucester, Massachusetts. (U.S.A.)

1. INTRODUCTION

The quality of chilled seafood reaching consumers is greatly dependent on the care taken in icing and handling on the vessel, use of sanitary conditions in processing and maintenance of refrigerated temperatures, as close to freezing as possible, from time of catch through all phases of processing and distribution, RONSIVALLI /1/. Previous research conducted by the Gloucester Laboratory of the National Marine Fisheries Service and other investigators has shown that temperature is a critical variable in the quality of fresh fish and that it is possible using the Arrhenius equation with activation energies of 18,000 cal/mole to predict the shelf life of chilled cod fillets, LEARSON and RONSIVALLI /2/, CHARM, et al /3/.

A rapid and practical objective means of measuring quality changes in chilled fish during distribution would be beneficial to producers, buyers, and personnel routinely carrying out product inspections. In certain packages, i.e., vacuum packs, where exposure to high temperatures for excessive periods of time could create safety problems, objective means of measuring time-temperature abuse could be very important in ensuring product safety, ECKLUND, et al /4/, WILHELM /5/.

Allied Corporation, U.S.A.^{1/}, has developed a computerized time-temperature monitoring system for measuring the freshness of semi-perishable and perishable foods during distribution. Studies were conducted at the National Marine Fisheries Service Northeast Fisheries Center Laboratory in Gloucester, Massachusetts, to evaluate this system in terms of relating the effectiveness of the monitoring system to the freshness of cod fillets that had been smoked, irradiated or dipped in potassium sorbate and stored at various refrigerated temperatures. This paper describes the experimental techniques used and reports the results of product freshness evaluation using the automated time-temperature monitoring system tests.

2. OBJECTIVE

- (1) To determine the shelf life and freshness of chilled cod fillets that were smoked, irradiated or dipped in potassium sorbate and stored at temperatures of 32, 42 and 52 degrees F.
- (2) To evaluate the effectiveness of the above automated time-temperature system in measuring and predicting the shelf life and freshness of chilled cod fillets processed as above.

3. EXPERIMENTAL

Description of the Automated Time-Temperature Monitoring System. The time-temperature monitoring system is composed of three principal parts:

- (a) A printed label incorporating a color changing chemical integrator;
- (b) A microcomputer with optical wand for reading the indicator label; and
- (c) Software for data analysis and telecommunications.

The Automated System incorporates proprietary color-changing polymers printed on labels in a bar code format. The indicator element is lightly colored at the start,

^{1/}Mention of Trade Names does not imply Endorsement by the NMFS, NOAA

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

but through the combined effects of time and temperature, its color intensity increases irreversibly. The rate of color development increases with increasing temperature.

The indicator labels (Figure 1) are comprised of two distinct types of bar codes. One is a standard bar code, either code 3 of 9 or interleaved 2 of 5, which provides information about the product's identity, date of manufacture, lot numbers or any other relevant inventory information. The other is an indicator code which contains a polymer that predictably develops color under different time-temperature conditions. These changes indicate cumulative temperature exposure that affects the product's shelf life. Polymer indicator labels are available for monitoring a wide range of perishable products.

A portable hand-held microcomputer (Figure 2) can read conventional bar codes as well as measure the reflectance of the polymer coating on the label. It has a total memory capacity of up to 80 kilobytes and can be downloaded to a remote micro or mini computer for permanent storage of the information and data analysis.

Figure 3 shows a schematic of the Automated System with labels on a shipping carton, portable microcomputer connected to a remote host computer via the commercial telephone network.

4. PREPARATION AND EVALUATION OF SAMPLES

Skinless cod fillets of known history were prepared as follows:

Irradiated at 200 kilorads, cold smoked or dipped in a 5% potassium sorbate solution. They were stored at temperatures of 33°F, 42°F and 53°F and evaluated by an expert taste panel using a 9 point hedonic scale, at periodic intervals of storage. Indicator labels were applied to individual packages and reflectance readings were taken during similar intervals of refrigerated storage. Particulars regarding specific treatment of samples were as follows:

Irradiated Cod Fillets. Fish were filleted one day after catching, stored at refrigerated temperatures; packaged in 2 mil polyethylene bags, irradiated at 200 kilorads and stored at temperatures of 33°, 42° and 53°F. Irradiation was performed with a cobalt 60 gamma irradiator at the University of Lowell two days after the fish were caught.

Sorbate Dipped Cod Fillets. Fish were obtained from the same lot as the irradiated fish and were handled similarly except that instead of being irradiated they were dipped in a cold fresh 5 percent potassium sorbate brine, placed in polyethylene pouches, heat sealed and stored at the different refrigerated temperatures.

Smoked Cod Fillets. Fish were obtained from a different lot than those above. filleted and smoked 3 days after catching, packaged in polyethylene pouches 7 days after catching and stored at various refrigerated temperatures. The fish were lightly smoked according to normal commercial techniques which consisted of immersion for 3 hours in a saturated sodium chloride brine solution and storage for 2 days in a 35°F refrigerated room prior to smoking. The smoked fish had a sodium content of 3 percent.

5. SENSORY ANALYSIS

Sensory tests were carried out at intervals of storage using an expert panel experienced in the evaluation of fishery product quality. A 9-point hedonic scale of Excellent (=9) to Inedible (=1) was used to rate the samples. Raw samples were rated for the attributes of appearance, odor and texture. Because of potential problems related to tasting processed fishery products stored at abusive temperatures the samples were not evaluated in the cooked state. The end of shelf life was determined when any of the sensory attributes fell below 5 on the 9-point scale.

6. INDICATOR LABELS

Indicator labels were attached to the outside of polyethylene pouch packs of irradiated, sorbate dipped and smoked cod fish fillets. A total of 180 indicator labels were used. Each label was scanned five times using the hand-held computer at periodic intervals of refrigerated storage. The readings stored in the hand-held

computer were loaded into a personal computer for evaluation. Reflectance readings were compared to taste panel findings. Computer estimated freshness measurements were made and compared with taste panel results using software developed by the Allied Corporation.

7. RESULTS

The sensory quality of irradiated, sorbate-dipped and smoked cod fillets at different times and temperatures of storage is shown in Figures 4, 5 and 6 respectively. For the smoked product the primary limiting factor was the appearance of the product, whereas odor was the limiting factor in the other samples.

The effect of storage temperature on shelf life of cod fillets subjected to different treatments is shown in Figure 7. It can be seen that the smoked fillets had a greater shelf life than irradiated or sorbate-dipped fillets, and the irradiated fillets had only slightly greater shelf life than sorbate-dipped fillets. As expected, higher temperatures resulted in a severe reduction of shelf life and had more impact on reduction of shelf life for the irradiated and sorbate-dipped fillets than for the smoked fish fillets. Figure 8 shows that temperature dependence of shelf life can be linearized using the Arrhenius relationship. Activation energies were determined to be about 15,000 cal/mole for the irradiated and sorbate treated samples and 5,000 cal/mole for the smoked samples.

Using the software supplied by Allied, Figure 10 shows the comparison of computer estimated shelf life versus the sensory analysis data for the irradiated fillets, and Figure 11 indicates the average and range of the computer estimated shelf life values.

8. CONCLUSIONS

The shelf life of smoked cod fillets was several days longer than irradiated or sorbate-dipped fillets with the limiting factor being appearance. With an increase in temperature, the shelf life of sorbate-dipped and irradiated fillets decreased more rapidly than did the shelf life of the smoked fillets. The Q10 values were 1.3 for the smoked fish and 2.8 and 2.6 for the irradiated and sorbate-dipped fish respectively.

Data on the irradiated fillets were analyzed in detail and results correlated with indicator label measurements. Reflectance readings of indicator labels were highly reproducible, and repeat runs were in close agreement. The standard deviation among individual indicator label reflectances was 2.5 percent. Averaged estimates of shelf life determined from indicator labels were consistently within 10 percent of measured values.

Results show that indicator labels could be used to satisfactorily monitor the freshness of irradiated fillets during distribution.

9. ACKNOWLEDGMENTS

These studies were carried out as part of a cooperative project between Allied Corporation and the National Marine Fisheries Service. We, particularly, acknowledge the assistance provided by Dr. Thaddeus Prusik and Mr. Paul Friedmann of Allied Corporation.

REFERENCES

1. Ronsivalli, L. J. A recommended procedure for assuring the quality of fish fillets at point of consumption. *Marine Fisheries Review*. Jan. 1982, Vol. 44(1).
2. Learson, R. J. and Ronsivalli, L. J. A new approach for evaluating the quality of fishery products. *Fishery Industrial Research*, Vol. 4(7). 1969.
3. Charm, S. E., Learson, R. J., Ronsivalli, L. J. and Schwartz, M. Organoleptic technique predicts refrigeration shelf life of fish food technology, Vol. 26(7). 1972.
4. Eklund, M. W., Poysky, F. T., Smith, C. A., and Reed, S. M. Outgrowth and toxin production of nonproteolytic Clostridium botulinum Types B, E, and F in

in nonirradiated and irradiated haddock fillets stored at 42° and 46°F. Final report No. TID-25750 to U. S. Atomic Energy Commission, Contract No. AT(49-7)-2442 - 44 pages. 1970.

5. Wilhelm, K. A. Extended fresh storage of fishery products with modified atmospheres: A survey. *Marine Fisheries Review*, Vol. 44(2). 1982.
-

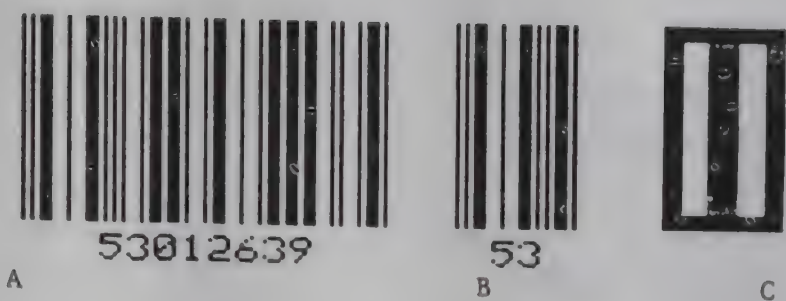
EVALUATION D'UN SYSTEME DE SURVEILLANCE AUTOMATIQUE DE LA DUREE ET DE LA TEMPERATURE POUR LA MESURE DE LA FRAICHEUR DU POISSON REFRIGERE

RESUME : La qualité des produits de la mer réfrigérés chez le consommateur dépend largement du soin apporté au glaçage et à la manipulation à bord du navire. Le respect de l'hygiène dans la transformation et du maintien d'une température, aussi voisine que possible de celle de congélation, dès la capture, et au cours de toutes les phases de la transformation et de la distribution est essentiel pour le maintien de la qualité et de la salubrité. Des recherches précédentes du "Gloucester Laboratory of the National Marine Fisheries Service" et d'autres chercheurs ont montré que la température était un paramètre essentiel de la qualité du poisson frais et qu'il était possible d'utiliser l'équation d'Arrhenius avec des énergies d'activation de 18 000 cal/mole pour prévoir la conservation en meuble de vente de filets de cabillaud réfrigérés.

Un moyen objectif, rapide et pratique, de mesure de l'évolution de la qualité du poisson réfrigéré pendant la distribution serait avantageux pour les producteurs, les acheteurs et des contrôleurs chargés des inspections de routine des produits. Dans certains emballages, comme les emballages sous vide, où l'exposition à des températures élevées pendant des périodes excessivement longues créerait des problèmes de salubrité, les moyens objectifs de mesure d'une durée et d'une température excessives seraient très importants pour être assuré de la salubrité du produit.

Ce rapport évalue l'utilisation d'un système de surveillance automatique de la durée et de la température, mis au point par "Allied Corporation", pour la mesure et la prévision de la qualité ou de la fraîcheur au cours de la distribution de filets de poisson emballés. Le système consiste en étiquettes, imprimées en format de code à barres, revêtues de polymères dont la couleur se modifie (passant du clair au foncé) sous l'influence combinée de la durée et de la température et en un micro-ordinateur portable pour lire la réflectance du revêtement de polymère et donner une mesure de la fraîcheur du produit à partir de la connaissance du couple temps-température.

Dans le présent essai, des filets de poisson fumés et irradiés ont été emballés et entreposés à diverses températures de réfrigération. Des étiquettes ont été appliquées à des emballages individuels de produit et à des cartons d'expédition. Au cours de l'entreposage, des essais de dégustation par un jury, des analyses microbiologiques et des lectures d'étiquettes ont été effectuées périodiquement. L'évaluation de la fraîcheur du produit par le système de surveillance automatique temps-température a été comparée aux résultats du jury de dégustation et des essais microbiologiques. Ce rapport examine la précision et la reproductibilité du système de surveillance automatique et son aptitude à prévoir la fraîcheur du produit.



- A User Specific Information
- B Freshness Identification Code
- C Freshness Bar Code

Figure 1
Freshness Label

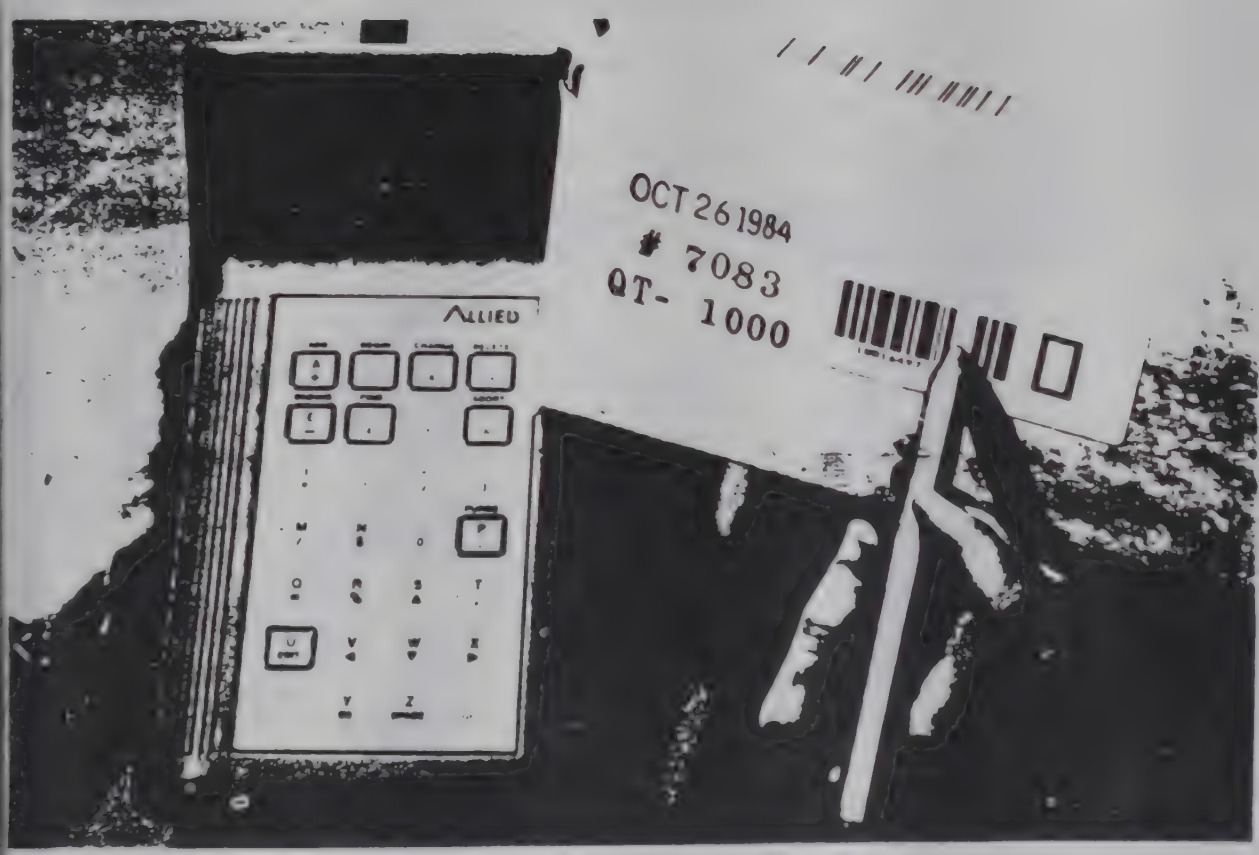


FIGURE 2
HAND-HELD COMPUTER

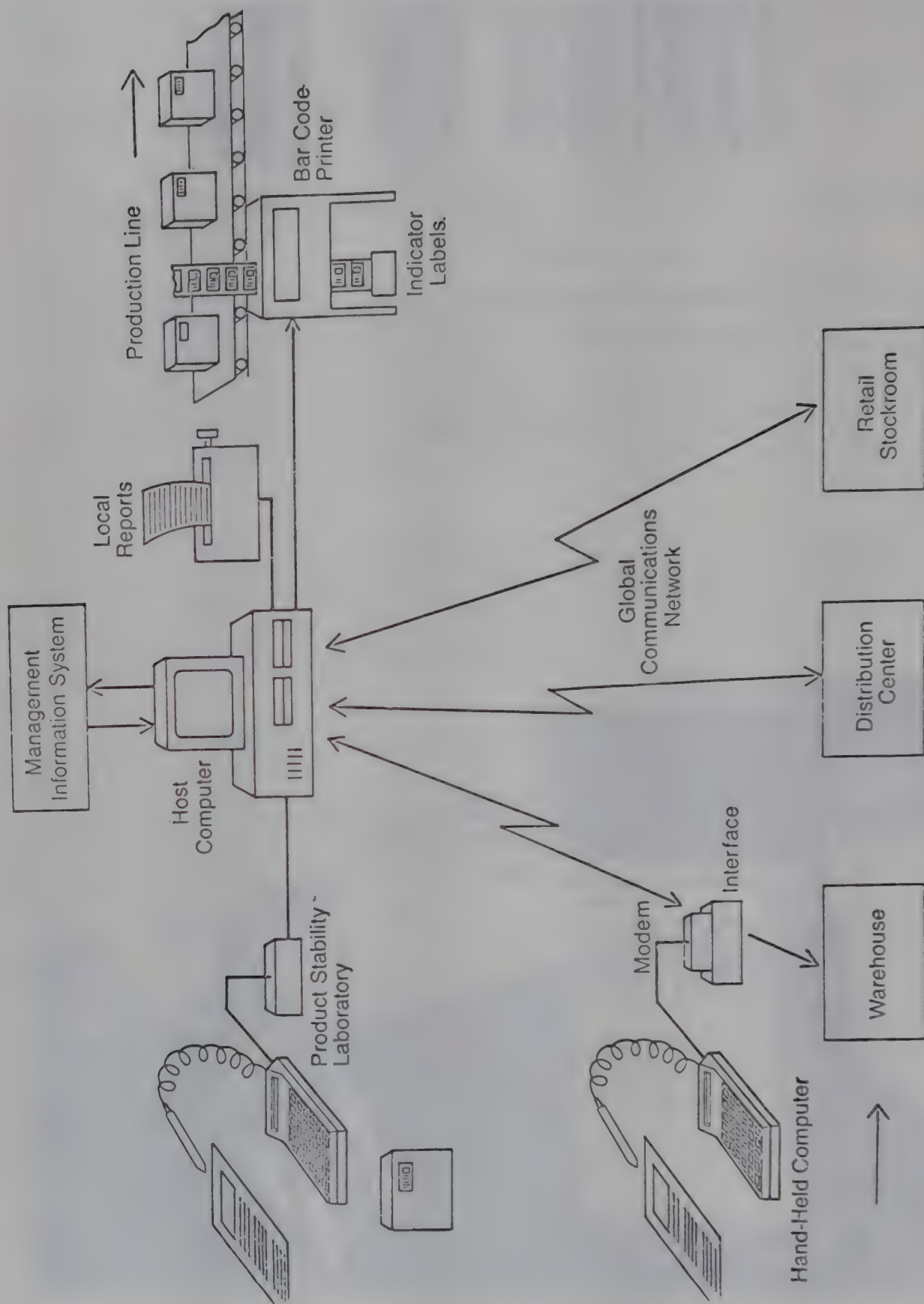
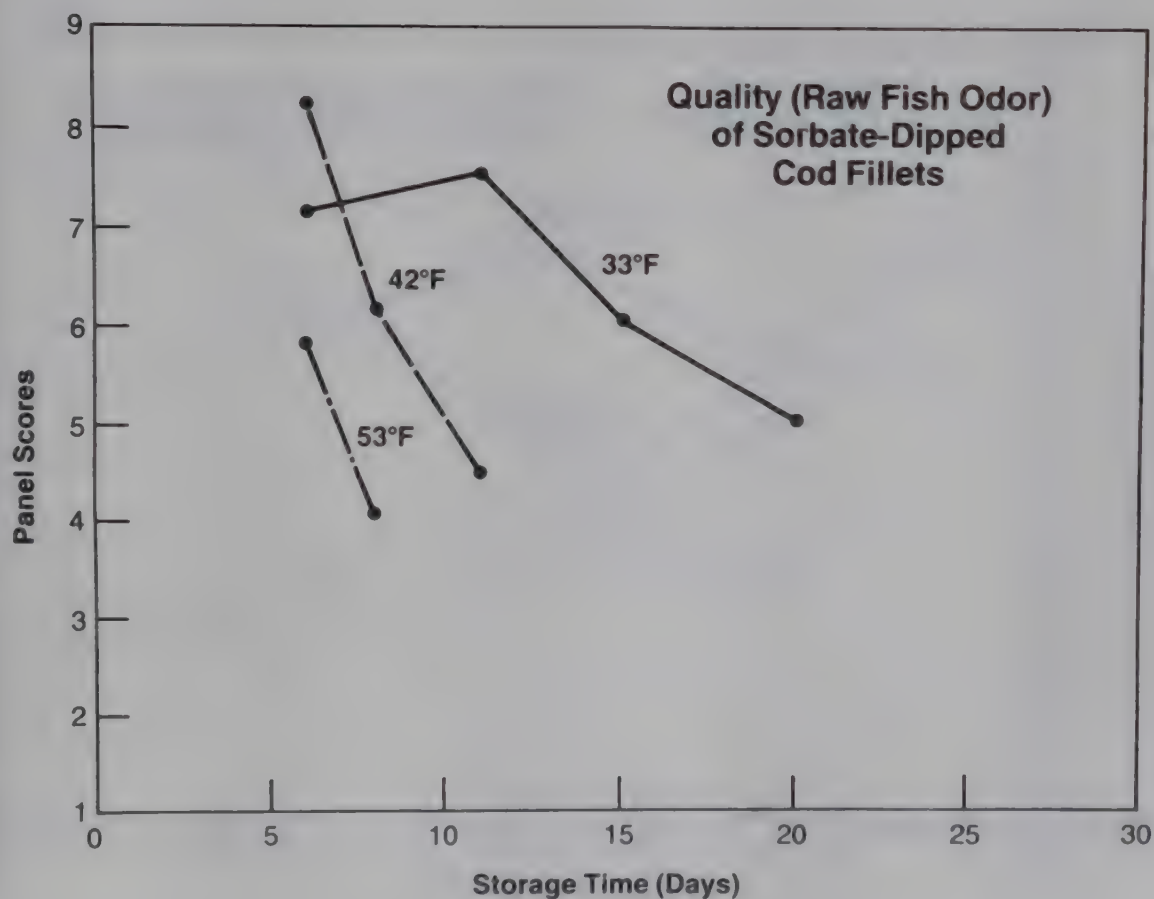
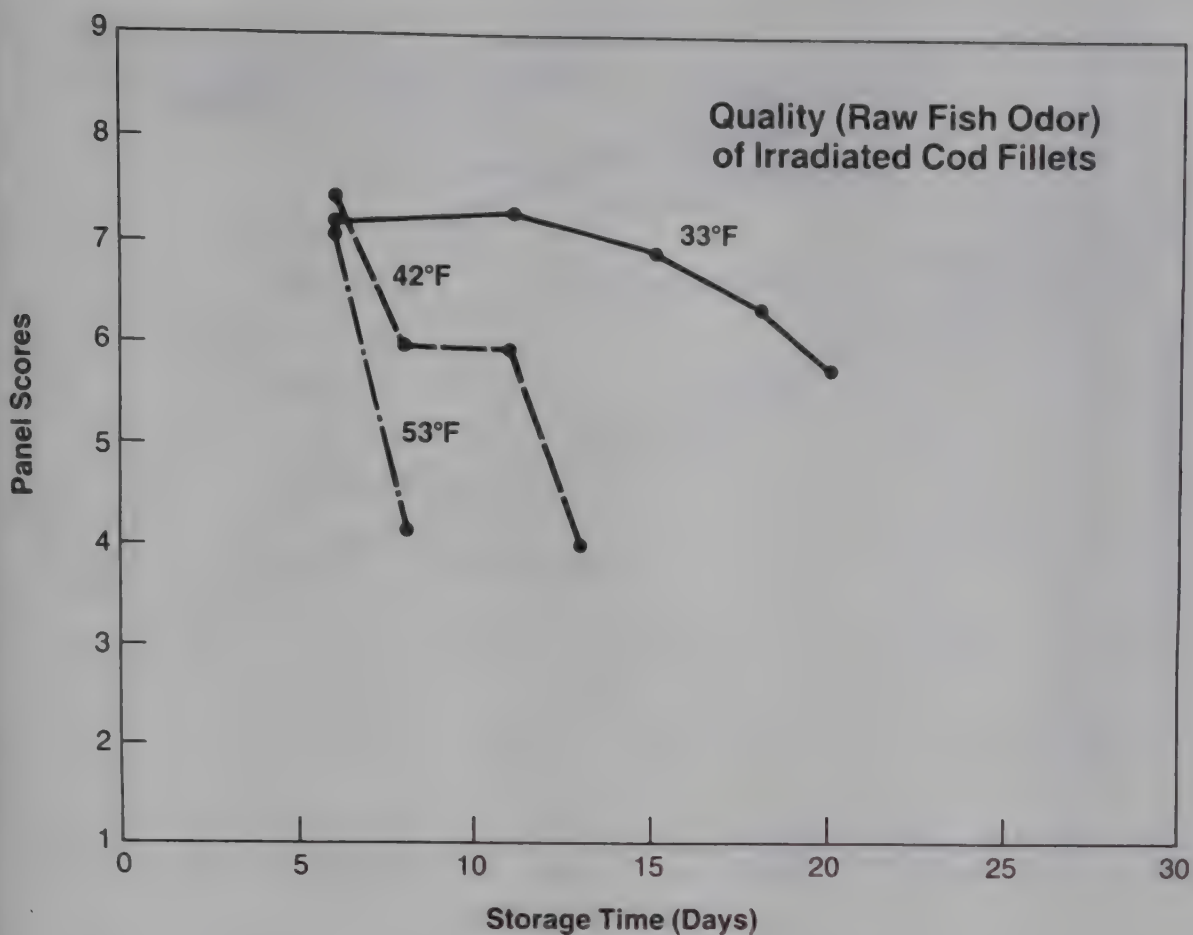
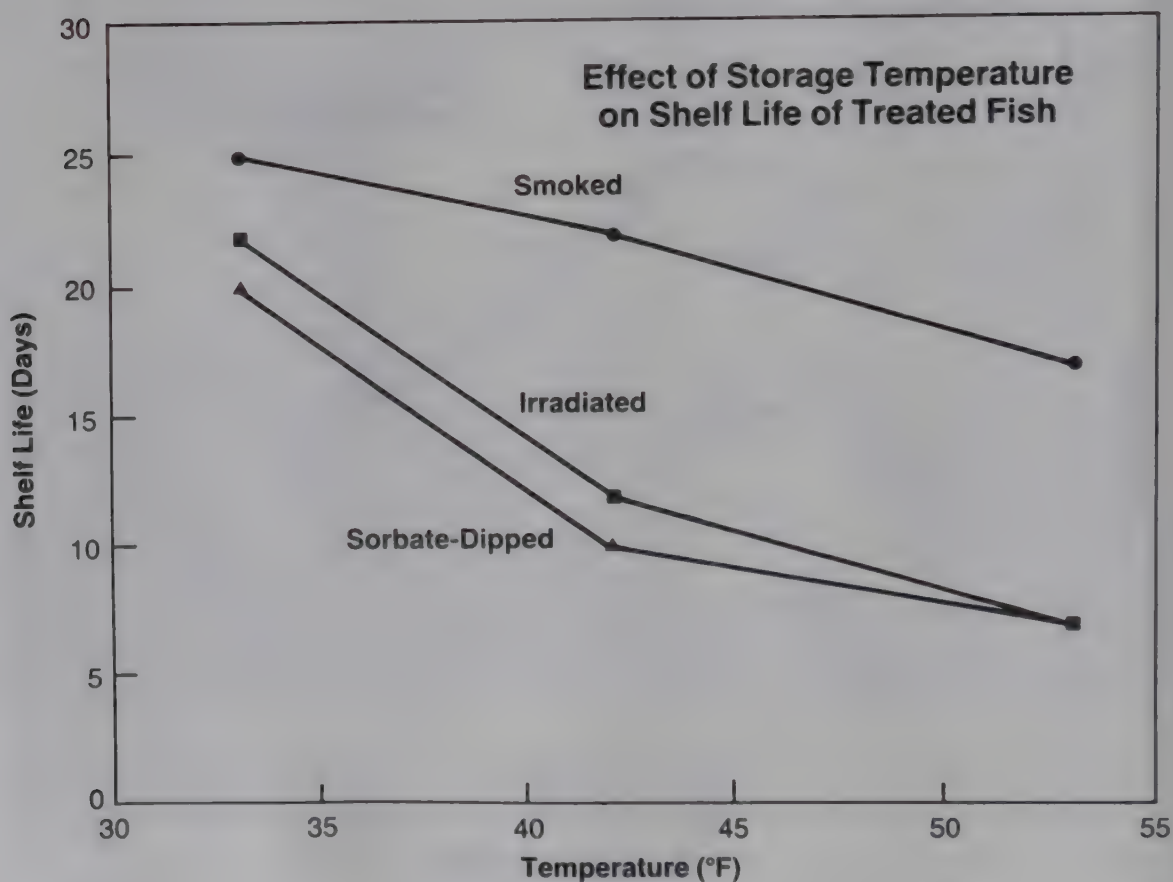
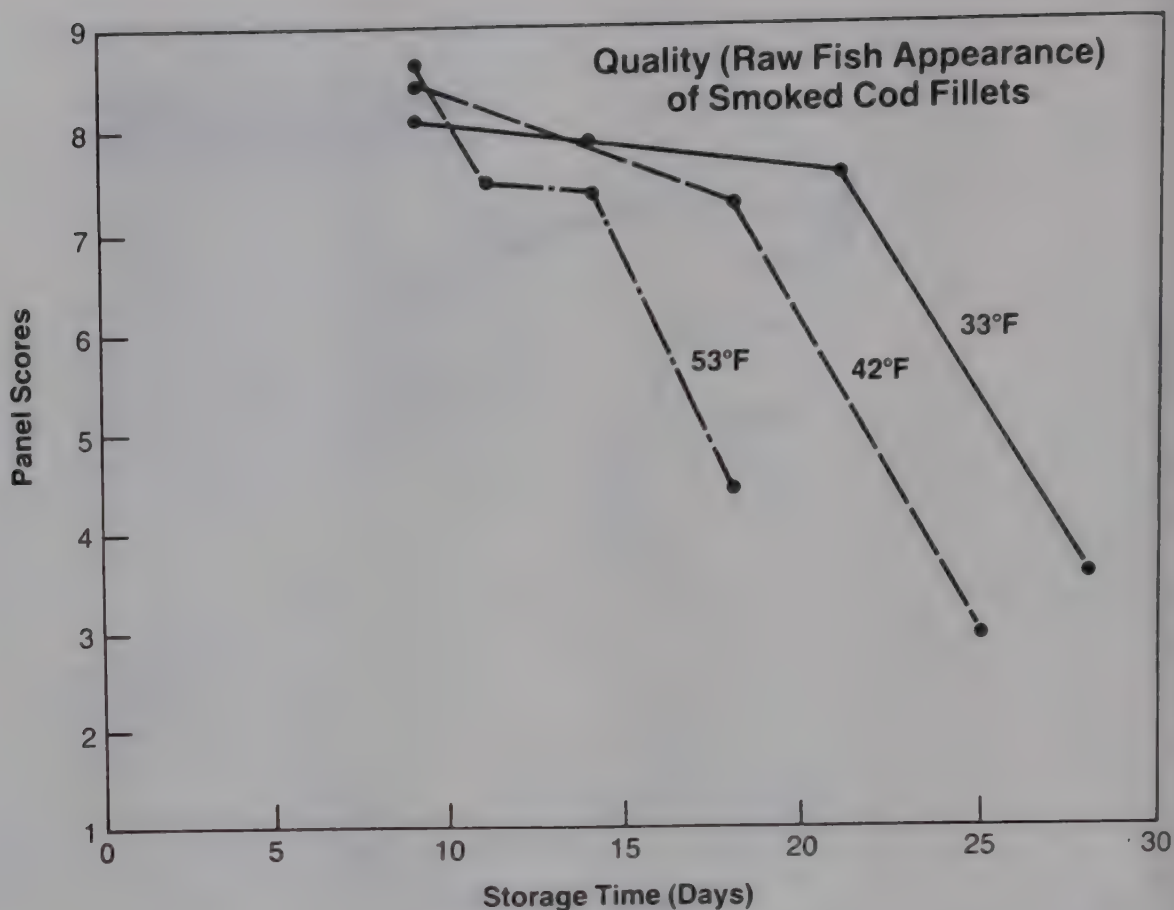
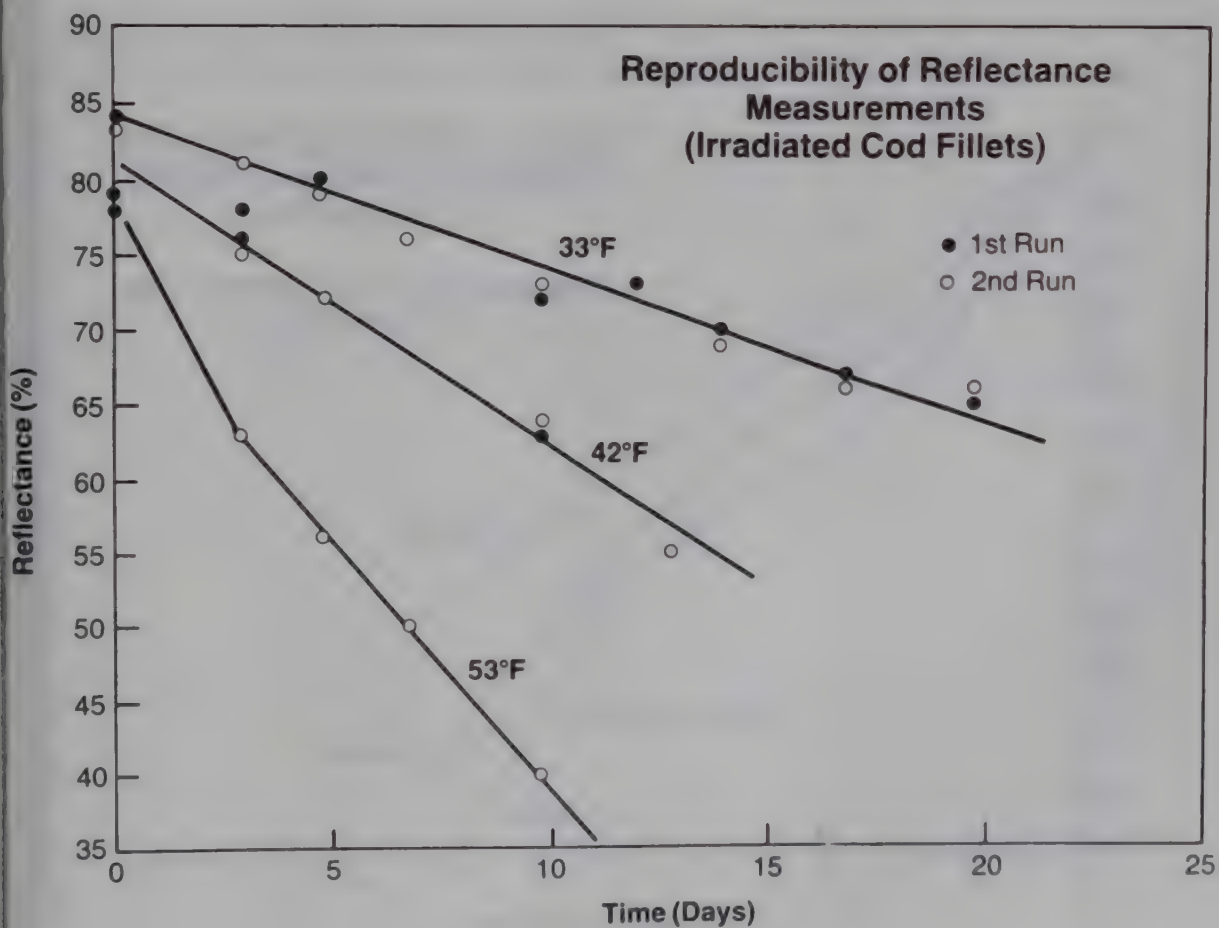
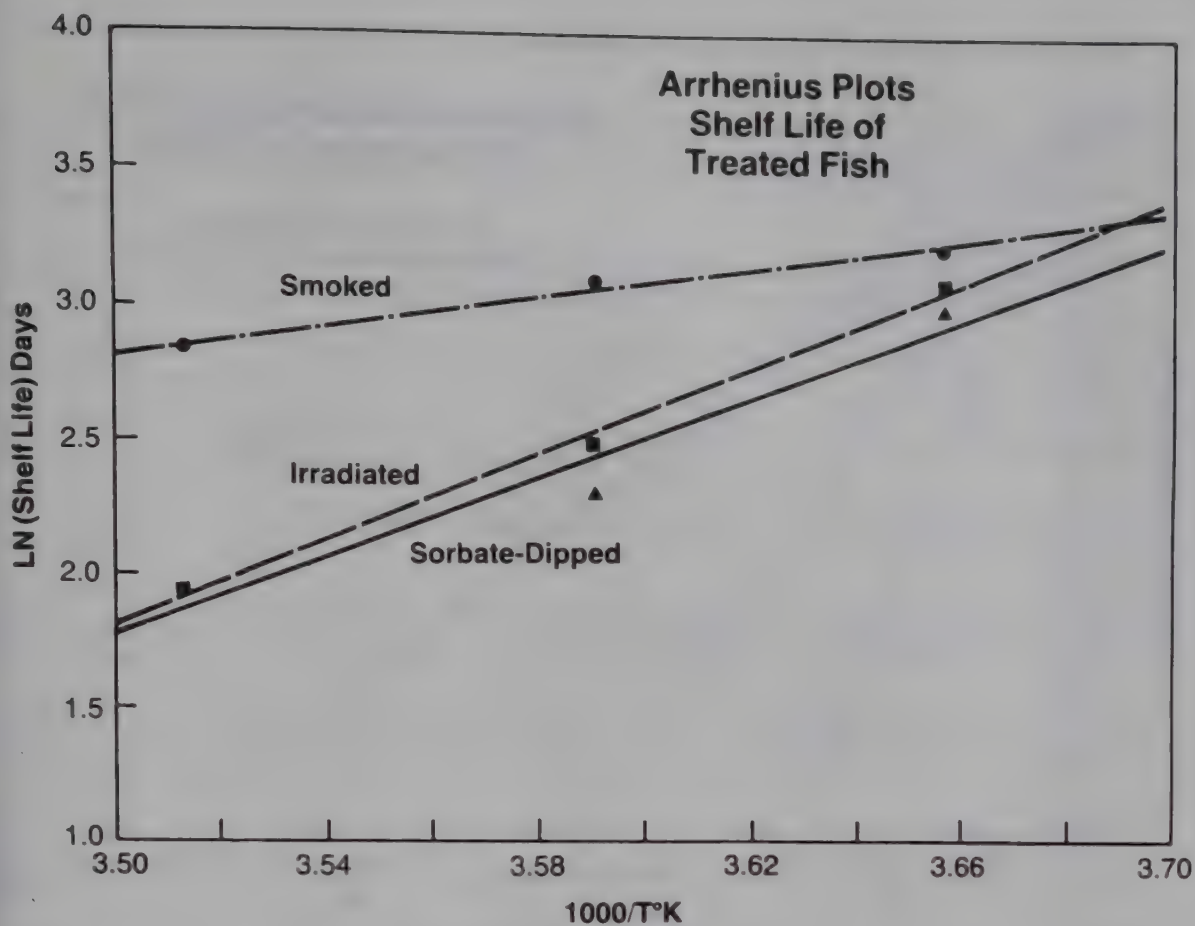


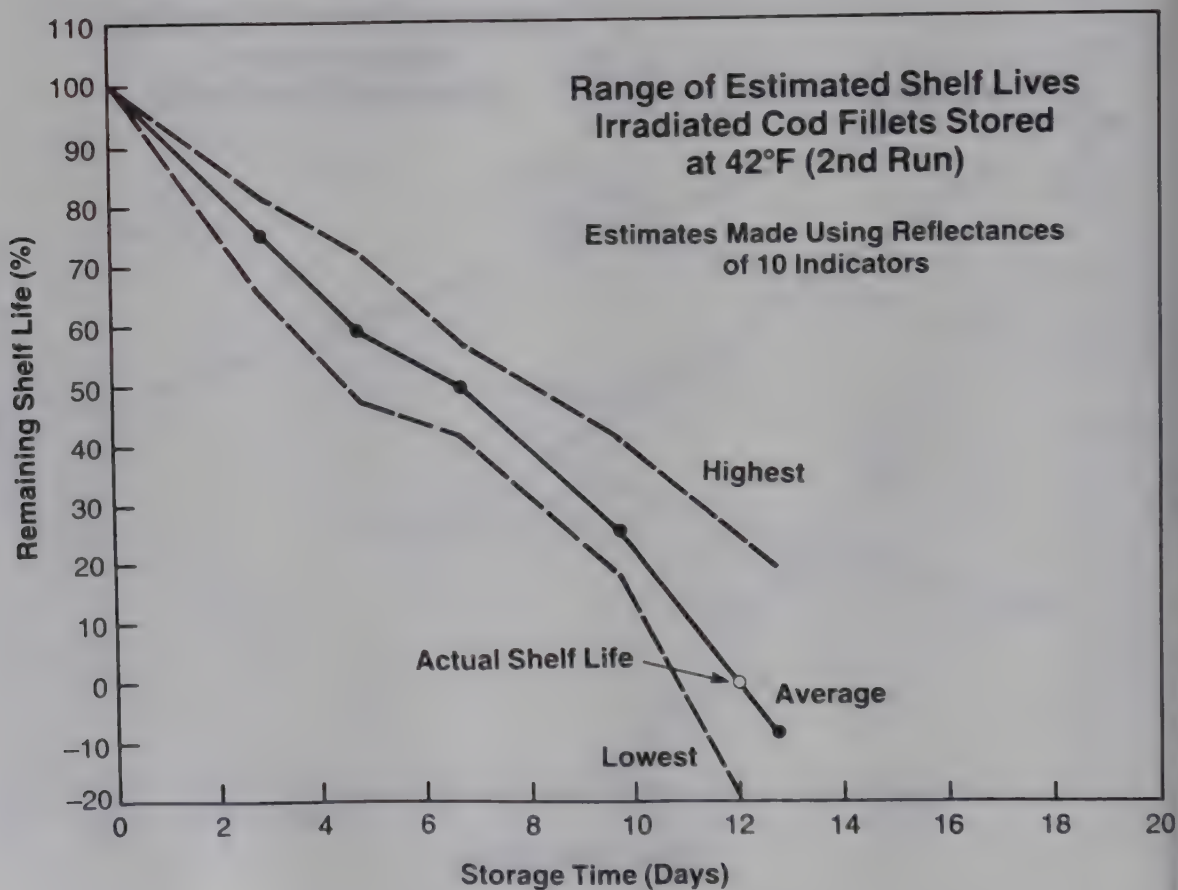
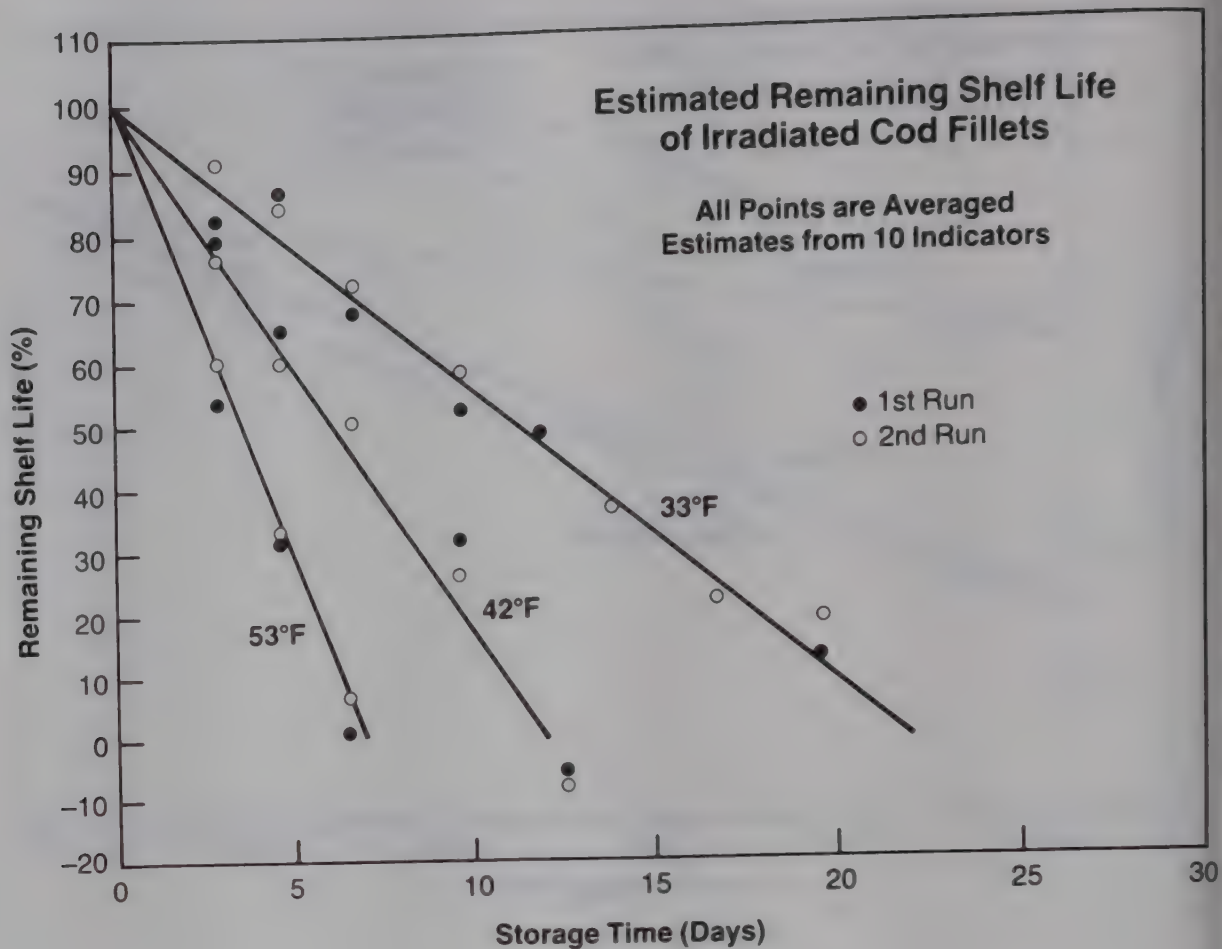
Figure 3

Lifelines Inventory Management System









DISCUSSION

J.W. JEBSEN (Norway) - Will cod, vacuum packed and irradiated with 200 k rad have a shelf life of more than 30 days when stored at 0°C ? The preservative effect, however, will not be due to the irradiation but to the enzymatic formation of formaldehyde ?

J.W. SLAVIN - Yes - you will note from the slides that irradiated fish stored at 33°F were still above the terminal point of quality when the test was ended.

K.J. WHITTLE (U.K.) - 1) Where were the T-T labels fixed - on isolated individual packs or on bulk stored packs ?

2) Does the monitor not tend to monitor the air temperature around the pack rather than the product temperature itself ?

J.W. SLAVIN - 1) The labels were placed on the outside of the polyethylene pouches containing the fillets.

2) We have found that the monitor registers the temperature of the fish. Even when the label is placed on the outside of the master carton, we find very close correlation with outside and inside readings.

A. BREMNER (Australia) - Can the time-temperature characteristics of the indicator strips be tailor made to suit different products that may have different time-temperature responses ?

J.W. SLAVIN - Yes. There are labels available for storage at ambient temperatures for months or years and storage of products at chill temperatures for weeks or months.

A. CORMIER (Canada) - Did you use a control sample in order to compare the sorbate, irradiation and smoke treatments ?

J.W. SLAVIN - Since the main purpose of the study was to evaluate the time-temperature monitoring system we did not use a control sample. Gloucester Laboratory is planning to carry out studies on non-treated chilled fish.

M. JUL (Denmark) - There has been a great deal of activity in recent years developing various label type, time-temperature integrator devices and the one described seems particularly interesting. Can you describe the time-temperature characteristics of the labels and also say what the approximate price per label unit would be?

J. SLAVIN - Yes; we have information on the characteristics of different labels and would be pleased to make these available to interested parties. The cost depends on the number required and at an annual requirement of 1 million labels costs would be between 10 and 15 U.S. cents each label. The labels are applied to master cartons.

TIME TEMPERATURE FUNCTION INTEGRATION, ITS REALISATION AND APPLICATION TO CHILLED FISH

R.M. STOREY
130, Carr Lane, Willerby, Hull, (United Kingdom)

1. INTRODUCTION

It is well accepted that temperature is the single most important factor which determines the shelf life of chilled fish products. Whereas there are many factors which can affect the rate of microbial spoilage, such as the degree of contamination by spoilage bacteria, species, season, processing prior to storage (e.g. filleting, skinning etc.) and packaging (e.g. storage in melting ice, vacuum packs and modified atmosphere packs), each of these factors is dominated by the effects of the storage temperature.

Storage temperature ideally should remain constant but close control of product temperature during the whole of its shelf life may be neither practicable nor economically viable. A prerequisite for marketing high quality chilled fish products is the capability of predicting shelf life with a reasonable degree of precision under the storage conditions to which such products may be subjected. Thus there is a requirement for some means of rapidly evaluating the effect on quality of the time temperature history which a product may have suffered. The degree of spoilage of a product is the only true integral of the effects of a fluctuating temperature history. However if the rate of spoilage at any given temperature as well as the time temperature history is known, the sum effect on spoilage of such a history can be computed. By continuously monitoring temperature and continuously summing the product of the rate of spoilage (computed from the measured temperature) and time over small time intervals, time temperature function integration is achieved. Such integrals can provide a measure of how much spoilage has occurred during the period of observation and thus how much shelf life has elapsed. The integral may be conveniently expressed in terms of an equivalent time at some reference, steady, temperature.

An essential requirement for time temperature function integration is the relationship between rate of spoilage and temperature.

2. RELATIONSHIP BETWEEN TEMPERATURE AND RATE OF SPOILAGE

For many years the rule of thumb that spoilage in chilled fish doubles for every 5 Celcius degrees rise in temperature has been applied, but Spencer and Baines /1/, Charm et al /2/ and Olley and Ratkowsky /3/, /4/ amongst others, have studied the effect of temperature on spoilage more closely. More recently Ratkowsky et al /5/ demonstrated that the square root of the growth rate of bacterial cultures was a linear function of temperature, up to temperatures at which the rate of growth began to decline, in contrast with the more usual exponential Arrhenius type of relationship. They proposed the following relation (eq. (1)),

$$\sqrt{r} = b(T - T_0) \quad (1)$$

Where b is the slope of the regression line, T is the temperature at which growth is measured and T_0 is regarded as a conceptual temperature. Mathematically T_0 is the value of T for which $r = 0$.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (U.K.) - 1985-4

The minimum temperature at which chilled fish is normally stored is 0°C, melting ice being an ideal refrigerating medium. It is convenient therefore to simplify eq. (1) and redefine growth rate r as the rate relative to that at 0°C. Eq. (1) thus becomes :-

$$\sqrt{r} = 0.1T + 1 \quad (2)$$

Where T is the temperature in degrees Celcius at which the growth rate is measured. Eq. (2) was found to agree closely with the general spoilage relationship proposed by Olley and Ratkowsky /3/, /4/ up to about 15°C, which suggests that eq. (2) is equally applicable to bacterial spoilage as it is to bacterial growth. More recently Ratkowsky et al /6/ have proposed a further empirical relationship for predicting the effect of temperature on growth rate over the range of temperatures at which bacteria grow.

Ratkowsky et al /5/ suggested that T_0 values (eq. (1)) for psychrotrophic bacteria are in the range 261 to 269°K, 270 to 280°K for mesophiles and above 290°K for thermophiles. Thus the constants, b and T_0 in eq. (1) can vary markedly for bacteria in the three major groups. Although this paper is primarily concerned with spoilage due to psychrotrophic bacteria, for which eq. (2) is applicable, sight should not be lost of the importance of the growth of organisms of public health significance, which tend to be mesophilic, and for which a modified equation (2) would have to be used.

3. PREDICTION OF RATE OF SPOILAGE

It is generally accepted that bacterial spoilage is the major reason for non-sterile chilled fish products becoming unacceptable to the consumer and whereas eq. (2) cannot be used to predict absolute rates of spoilage (or absolute rates of bacterial growth) with increase in temperature, it can be used with acceptable precision to predict increases in the rate of spoilage relative to that at 0°C for a particular product stored under a particular set of conditions. This can be illustrated by considering the spoilage data reported by Cann et al /7/. These authors carried out extensive chilled spoilage studies at 0, 5 and 10°C on a variety of fish products stored either in vacuum packs or in a modified atmosphere of 40% CO₂, 30%N₂ and 30%O₂. Bacteriological, chemical and sensory analyses were made at appropriate time intervals. Cann et al's data for cod and herring fillets stored in vacuum packs and modified atmosphere packs have been analysed to compare actual rates of spoilage at 5 and 10°C with those predicted from the measured spoilage rate at 0°C, using eq. (2). Regression equations of days on cooked flavour score have been calculated for each product stored under both conditions and the number of days required for the cod to decrease to a cooked flavour score of 6 on the Torry 10 point objective white fish scoring system (10 = fresh, 0 = putrid) calculated. For herring the time for the flavour score to rise to 3 on the Torry 6 point herring scoring system (1 = fresh, 6 = repulsive, Table 2 - 5, p. 27 ref. /7/) was determined. The results are given in Tables 1 and 11 and it can be seen that in all cases the predicted times are within the 95% confidence limits of the measured spoilage times. Similar results were obtained for total viable counts.

The evidence presented in Tables 1 and 11 overleaf suggests that, provided information is available on the shelf life for a particular product at 0°C (or some other known temperature), then the shelf life at other temperatures can be predicted with an adequate precision, which cannot of course be greater than the precision of the data upon which it is based.

TABLE I

Comparison of measured and predicted times for cod fillet spoilage

Temperature °C	Modified Atmosphere Packs			Vacuum Packs		
	Days to *CFS 6	95% CL **(Days)	Predicted Days	Days to CFS 6	95% CL (Days)	Predicted Days
0	12.7	2.6	-	7.8	2.2	-
5	4.5	1.4	5.6	3.5	2.3	3.5
10	2.4	1.2	3.2	2.2	1.1	1.9

* Days to cooked flavour score = 6

** 95% Confidence limits.

TABLE II

Comparison of measured and predicted times for herring fillet spoilage

Temperature °C	Modified Atmosphere Packs			Vacuum Packs		
	Days to *CFS 3	95% CL (Days)	Predicted Days	Days to CFS 3	95% CL (Days)	Predicted Days
0	7.3	3.9	-	9.2	6.3	-
5	3.2	1.4	3.2	3.5	1.1	4.1
10	2.1	0.4	1.8	2.0	0.5	2.3

* Days to cooked flavour score = 3

Although space does not allow the presentation of further data (but see for example, Olley & Quarmby /8/), one further question remains unanswered. Do fluctuating storage temperatures per se have an effect on rates of spoilage? Charm et al /2/, who stored cod fillets under various time temperature regimes within the temperature range 1 to 8°C, concluded that taste panel scores were independent of the order in which temperature variations occurred during storage. Their results suggest that, for the range of temperatures studied, temperature fluctuation itself did not contribute to spoilage. Spencer (Spencer & Baines /1/) also obtained data on the spoilage of whole gutted cod stored under a fluctuating temperature regime, but unfortunately this work has not been published. However when Spencer's time temperature data (he stored fish at +1°C, +15°C and also interchanged fish samples between +1 and +15°C every 12 hours until the fish became spoilt) is converted to equivalent time at 0°C, using equation (2), and the results plotted against taste panel scores, all the points fall on a common line, the slope of which, 0.323 (reduction in taste panel score per day at 0°C) is very similar to a value (0.334) proposed by Baines and Shewan /9/ for iced fish. Although definitive data is sparse, what is available suggests that the effect of fluctuating temperatures per se on spoilage, as measured by a taste panel, if any, is small.

Thus simple integration of time and temperature function can provide a useful indication of spoilage, provided that data at some known temperature is available, preferably, but not essentially at 0°C.

4. TIME TEMPERATURE FUNCTION INTEGRATION

Integration can be carried out manually from time temperature records of course, but is cumbersome, time consuming and integrals indicating spoilage or loss of shelf life are only available after the event. Small portable data loggers such as the "Squirrel" (Grant Instruments Ltd.) and the "Autolog" (Remonsys Ltd.) store data which can be readily retrieved directly into a computer. Instruments are available which integrate continuously and display the integral as an equivalent time at an appropriate temperature, such as those used in the canning industry for the determination of F_0 values. These are usually expensive and not portable. A portable instrument designed specifically for monitoring fish spoilage, is the "Tefimupot" (New Zealand Development Authority, US Patent 4061033) which is rather expensive, but does provide a continuous readout of equivalent time at 0°C.

No entirely suitable instrument is yet available so far as is known. The author devised a simple instrument based on a digital watch integrated circuit ("chip"), in an attempt to produce a small, robust and cheap direct reading integrator. A thermistor senses temperature and provides a signal, suitably processed by the circuitry, to drive the watch at relative rates defined by equation (2). The watch reads equivalent time at a selected reference temperature, e.g. 0°C. For example, for a steady temperature of 10°C the watch would read 4 days expired time in a real time of 1 day. By slowing the watch to 1/24 of its normal speed, a 24 hour watch will integrate over a period of 24 days (at 0°C, 24 days equivalent at other temperatures). This instrument is capable of further development, but as yet is commercially available only in basic form. A more complex version of the device has been described by Owen & Nesbitt /10/.

The cost of integrators can be greatly reduced by the development of dedicated integrated circuits. Ultimately it may be possible to produce silicon based integrated circuits in which all the circuitry, including the sensor and visual display, will be contained on the "chip", but this will be very much dependent on market demand.

5. CONCLUSIONS

The major aim of this paper has been to draw attention to the potential value of time temperature function integration as a means of predicting spoilage during chilled storage under conditions of fluctuating temperature and thereby providing an indication of loss of shelf life.

Time temperature function integrals (equivalent times) are often greater (longer) than may be apparent from a cursory inspection of time temperature records. For example finite times are required to cool fish products to the desired storage temperature; the equivalent times at the storage temperature can be three or four times longer than the measured time required to cool the product and may be a significant proportion of the total storage time. As a final example, some 25 years ago Torry Research Station carried out an extensive survey /11/ of the temperature of iced fish during distribution from fishing port to retail outlet. Appropriate integration of the time temperature data indicates that the equivalent time at 0°C was 200 hours compared to the observed average time of 38 hours for distribution. This suggests that the actual spoilage was over five times greater than that that would have occurred if the fish had been at 0°C for the whole of the time!

The ideal integrator for instant display of expired shelf life is perhaps not yet available. Even when and if such a device becomes available, although it may be re-used many times and may possibly be more precise than biochemical or chemical integrators, it will almost certainly be more expensive than the latter. Perhaps those responsible for distributing chilled fish should initiate development of a suitable device.

REFERENCES

1. R. SPENCER AND C.R. BAINES : The effect of temperature on the spoilage of wet white fish. Food Technol 18, 769-773, (1964).
2. S.E. CHARM, R.J. LEARSON, L.J. RONSIVALLI AND M. SCHWARTZ : Organoleptic technique predicts refrigeration shelf life of fish. Food Technol. Champaign, 26, 65-68, (1972).
3. J. OLLEY AND D.A. RATKOWSKY : Temperature function integration and its importance in the storage and distribution of flesh foods above the freezing point. Food Technol. Aust. 25, 66-73, (1973).
4. J. OLLEY AND D.A. RATKOWSKY : The role of temperature function integration in monitoring of fish spoilage. Food Technol. N.Z. 8, 13, 15 & 17. (1973).
5. D.A. RATKOWSKY, J. OLLEY, T.A. McMEEKIN AND A. BALL : Relationship between temperature and growth rate of bacterial cultures. J. Bact. 149, 1-5, (1982).
6. D.A. RATKOWSKY, R.K. LOWRY, T.A. McMEEKIN, A.N. STOKES AND R.E. CHANDLER : A model for the rate of growth of bacterial cultures throughout the entire biokinetic range. J. Bact. 154, 1222/1226, (1983).
7. D.C. CANN, G.L. SMITH AND N.C. HOUSTON : Further studies on marine fish stored under modified atmosphere packing. Ministry of Agriculture Fisheries and Food, Torry Research Station, Aberdeen (Undated).
8. J. OLLEY AND A.R. QUARMBY : Prediction of storage life at higher temperatures, based on storage behaviour at 0°C, and a simple visual technique for comparing taste panel and objective assessments of deterioration. Trop Sci. 23, 147-153, (1981).
9. C.R. BAINES AND J.M. SHEWAN : Taste panel evaluation of fish quality. Lab. Practice, 14, 160-162 (1965).
10. D. OWEN AND M. NESBITT : A versatile time temperature function integrator. Lab. Practice, 33, 70-75 (1984).
11. G.H.O. BURGESS, R.M. COCKBURN, C.L. CUTTING AND W.B. ROBB : The temperature of British fish during distribution in Summer. Torry Technical Paper No. 1, H.M.S.O., Edinburgh (1959).

INTEGRATION DE LA FONCTION TEMPS TEMPERATURE, SA REALISATION ET SON APPLICATION AUX POISSONS REFRIGERES

RESUME : La température est, à elle seule, le facteur le plus important pour déterminer la durée de conservation de poissons (et produits dérivés) réfrigérés; elle peut prévaloir sur d'autres facteurs, également importants, tels que la préparation et l'emballage.

Des variations de température pendant la préparation, l'entreposage et la distribution ne peuvent pas être évitées en réalité; c'est pourquoi la façon d'évaluer ces effets pendant l'entreposage est une aide précieuse pour le spécialiste du poisson.

On examine les concepts de l'intégration de la fonction temps-température et l'expression des intégrales en termes d'équivalence de temps à une température constante définie. On cite également une fonction de température convenant à l'intégration. Les méthodes d'intégration sont brièvement analysées.

SECTION VIII

PRÉSENTATION PAR AFFICHES

POSTER PRESENTATIONS

ICED STORAGE LIFE OF TWO FISH SPECIES FROM VANUATU

G R Ames and C A Curran

Tropical Development and Research Institute
London WC1X 8LU (United Kingdom)

BACKGROUND

Most of the 80 islands of Vanuatu are very fertile and until recently fishing has not been a major activity. Now village fishing cooperatives are being set up to supply the growing tourist trade and to provide fish for export. Studies of the fishery potential have shown a significant resource of deep-water fish on the reef slopes. The most prevalent species are *Pristipomoides* and *Etelis*, especially *P. multidens* and *E. carbunculus* all of the family Lutjanidae. These species are very popular with residents and visitors, and they are known collectively as "poulet"; in an area where many species can cause ciguatera poisoning, these are considered "as safe to eat as chicken".

MATERIALS AND METHODS

Pristipomoides multidens and *Etelis carbunculus* were caught off Port Vila by long line, at depths of about 200 m. On being brought into the boat they were killed by the ika jimi technique (spiking through the brain) and placed in an ice/water slurry. After landing they were packed in flake ice in fish boxes which allowed drainage of melt water. The boxes were kept in a chill room maintained at 1-3°C. More ice was added as necessary, and the fish were repacked in fresh ice once a week. Care was taken to handle the fish as little as possible, and when it was necessary to touch them, they were held only by the head and tail.

Samples were taken twice a week for examination, four fish for *P. multidens* and two for the larger *E. carbunculus*.

Taste panel. Fillets were steamed for 15 minutes and presented to a panel of 6-12 people. Most of the panel members were expatriates resident in Vanuatu, very familiar with these types of fish. Scoring was on a five point scale.

5. Excellent: very fresh flavour and odour
4. Good: fresh flavour and odour
3. Acceptable: neutral flavour and odour
2. Poor but acceptable: slight off-flavour or odour
1. Unacceptable: strong off-flavour or odour.

The minimum level of acceptability was set at an average score of 1.5.

In addition to a visual and olfactory assessment, the following measurements were made:

Torrymeter reading: the mean of 16 readings taken from the right side of the fish.

Total plate count on skin and flesh: 25 cm² of skin and 10 g of flesh were removed aseptically from the left side of the fish. These were mixed with bacteriological peptone solution and then a tenfold dilution series was inoculated onto plate count agar. The plates were incubated for three days at room temperature (28-32°C) and the colonies counted.

pH of the minced fish muscle: two samples were dispersed in 0.05 M sodium iodoacetate solution and the pH was measured with an EIL portable pH meter.

Total volatile bases in the minced fish muscle, using the Conway method. /1/

A carcass analysis was carried out on ten fish of each species.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

TABLE I CARCASE ANALYSES

	Range	Mean
<i>Pristipomoides multidens</i>		
Total weight, g	342 - 3060	987
Fillet, %	28.8 - 44.0	37.4
Skin, %	4.6 - 10.7	7.3
Guts and gills, %	5.8 - 10.1	7.6
Head and frame, %	41.8 - 55.4	47.7
<i>Etelis carbunculus</i>		
Total weight, g	680 - 3050	1354
Fillet, %	32.6 - 38.8	36.5
Skin, %	6.1 - 9.0	7.5
Guts and gills, %	5.1 - 10.3	7.7
Head and frame, %	42.4 - 50.3	48.3

TABLE II VISUAL AND OLFACTORY EXAMINATION

		noted on day	
	Change to	<i>P. multidens</i>	<i>E. carbunculus</i>
Eyes	Sunken, cloudy	10	14
	Slightly bloody	16	25
	bloody	23	-
Gills: odour	Oniony	13	14
	Sulphury, unpleasant	16	18
	Faecal	23	25
Gills: appearance	Red, clear mucus	7	5
	Brown, brown mucus	23	21
Skin	Less bright	10	14
	Rather dull	20	21
	Dull	28	25
	Some loose scales	35	28
Flesh	Fairly firm	16	11
	Softer but still resilient	31	21
Belly cavity*	Distended	31	28

* By the time the fish had been brought to the surface the belly contents had been forced into the mouth, leaving the cavity empty.

RESULTS

After one - two weeks both species of fish became bland and lacking in flavour. Then, after two weeks (*E. carbunculus*) or three weeks (*P. multidentis*) the taste panel found a slight improvement in flavour, presumably as slight off-flavours had a beneficial effect. After a further week, the taste panel scores dropped. However, the storage life of both species is very long. *E. carbunculus* was slightly above "poor but still acceptable" after 4 weeks, when the last sample was examined. *P. multidentis* was "acceptable" after 31 days, and it was still above the rejection level after 35 days.

The TVB measurements (Fig. 2) were in accordance with the taste panel results. The TVB content of *P. multidentis* showed a general increase to 37 - 38 mg N/100 g after five weeks; for *E. carbunculus* it reached 30 mg N/100 g after four weeks. Usually 35 - 40 mg N/100 g is regarded as the maximum level of acceptability /2/.

The total plate counts of bacteria (Fig. 3 and 4) on the skin reached 10^8 at about the time when the taste panel found the fish to be unacceptable. The plate counts for the flesh increased in line with the skin counts, but the bacterial levels in the flesh were only 1/50 - 1/100 those on the skin. For some other species, the bacterial levels in the flesh are about 1/10 those on the skin /3/.

The storage life of these species is unusually long. Relatively few tropical species, even when handled under ideal conditions can be stored for as long as 3-4 weeks /4/, but these species remain acceptable for 4-5 weeks. Bacterial growth is usually the limiting factor in iced storage, so the relatively slow growth of bacteria on the skin of these species, and the lower levels in the flesh, appear to be the reason for the long storage life.

It may be noted that the storage conditions did not allow any significant washing of the skin of the fish by ice water.

CONCLUSIONS

Pristipomoides multidentis and *Etelis carbunculus* have very long storage lives in ice: five weeks and at least four weeks respectively. This will greatly facilitate the commercial exploitation of the species in Vanuatu and other parts of the Pacific region.

Frozen storage trials of *Pristipomoides multidentis* are being carried out at the TDRI.

REFERENCES

1. Beattie, S.A. and Gibbons, N.E: The measurement of spoilage in fish. J. Biol. Bd. Can. 1937, 3 (1) 77-91.
2. Connell, J.J: Control of fish quality, Surrey: Fishing News (Books) Ltd (1975) 179 pp.
3. Curran, C.A., Crammond, V. de B. and Nicolaidis, L: Spoilage of fish from Hong Kong at various temperatures. Trop. Sci. 1981, 23 (2) 129-145.
4. Lima dos Santos, C.A.M.: Storage of tropical fish in ice - A review. Trop. Sci., 1981, 23 (2) 97-127.

Fig. 1

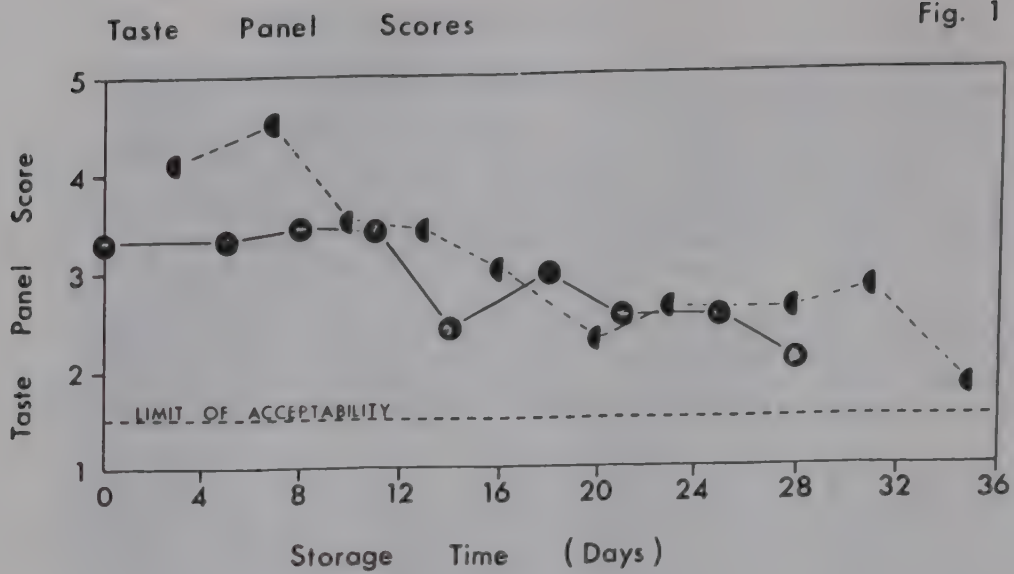
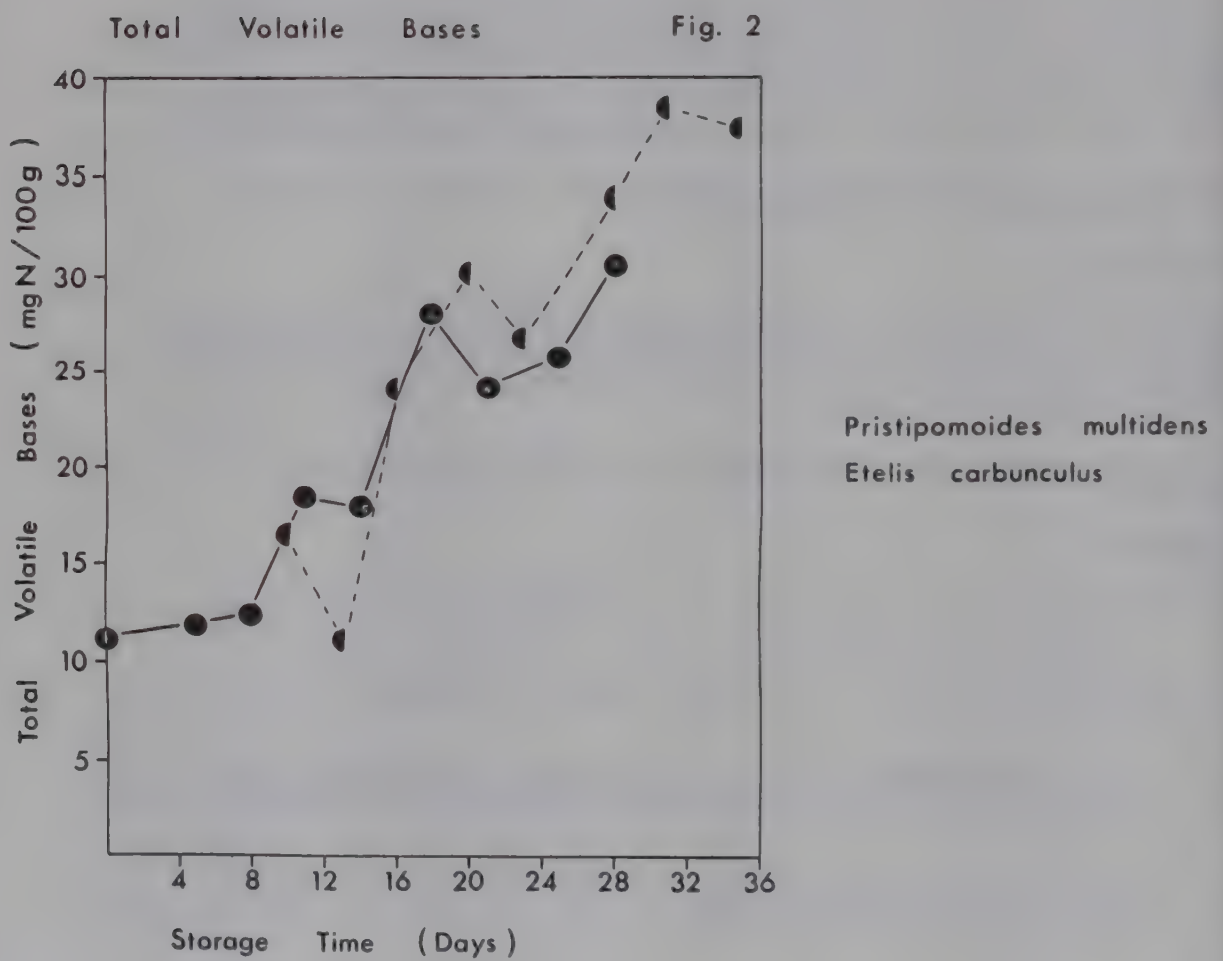
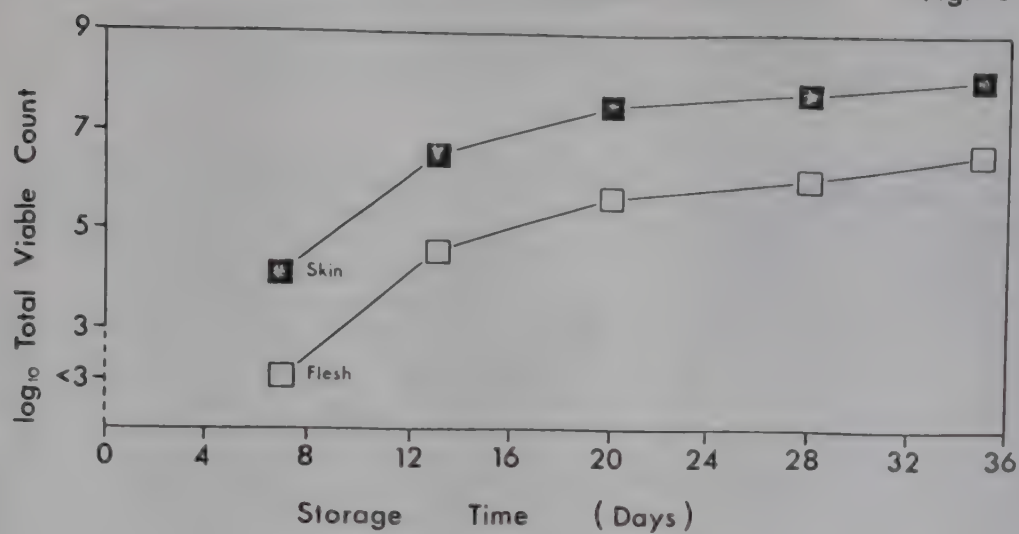


Fig. 2



Total Viable Count : *P. multidens*

Fig. 3



Total Viable Count : *E. carbunculus*

Fig. 4

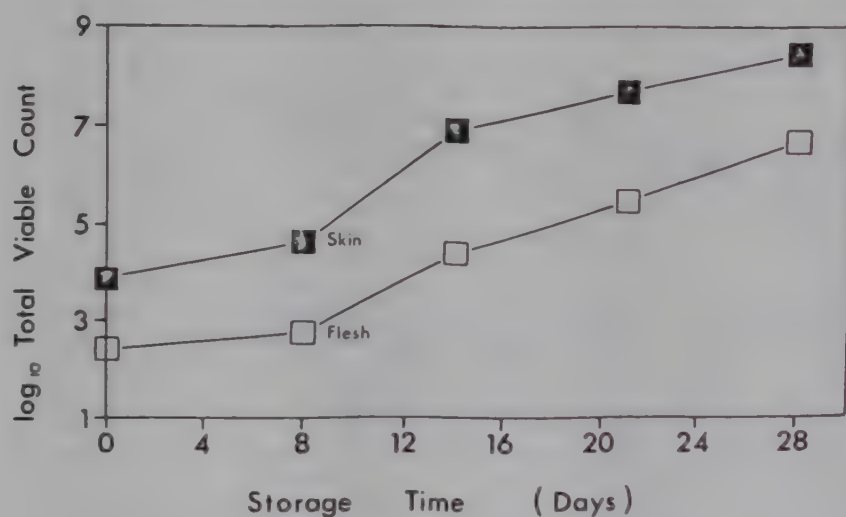


Fig. 5

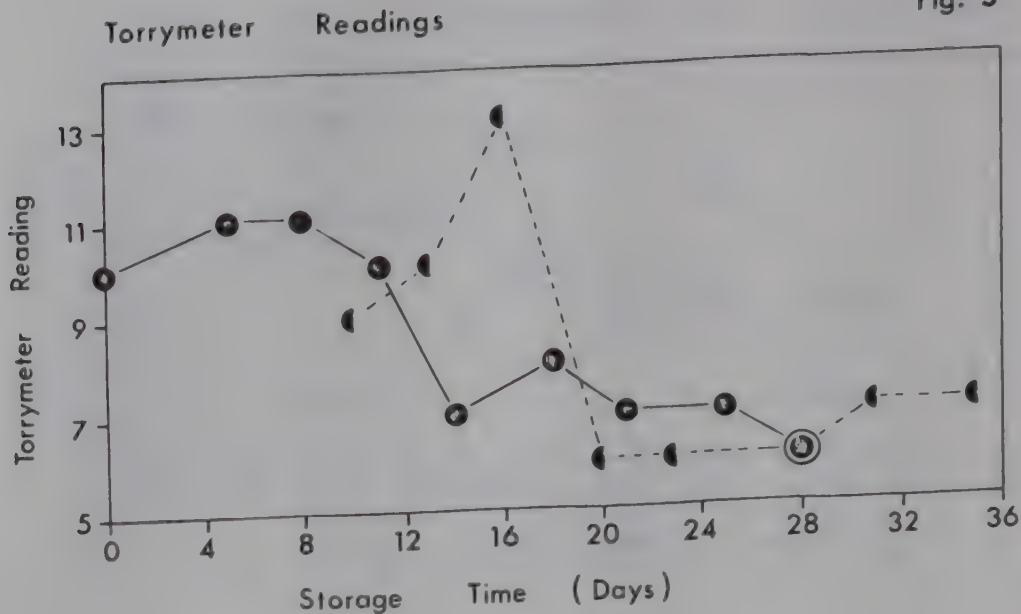
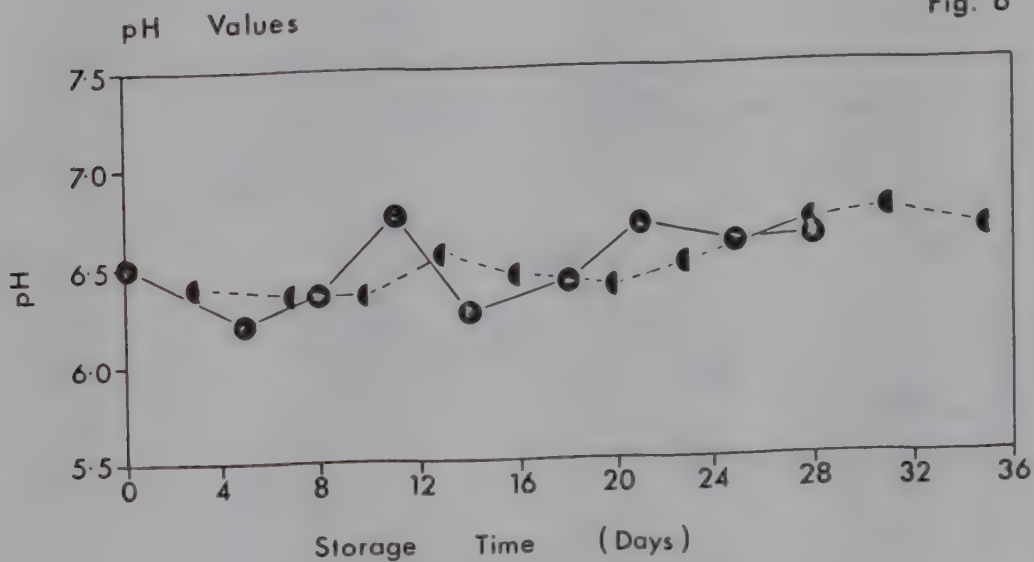


Fig. 6



DUREE DE CONSERVATION DANS DE LA GLACE DE DEUX ESPECES DE POISSON DE VANUATU

RESUME: Des essais de conservation dans la glace ont été réalisés sur deux espèces de poisson de Vanuatu : le Pristipomoides multidens et l'Etelis carbunculus, des lutjanidés d'eau profonde. Après une conservation de 35 jours dans de la glace, un jury de dégustation a trouvé que le Pristipomoides multidens était acceptable. Au bout de quatre semaines, le jury de dégustation a trouvé que l'Etelis carbunculus était encore acceptable lorsque le dernier des échantillons a été examiné. Après ces durées, les niveaux bactériens sur la peau et dans la chair et la teneur totale en bases volatiles de la chair ont atteint des valeurs indiquant en général l'inacceptabilité.

Cette longue durée de conservation inhabituelle dans de la glace facilitera énormément le développement de l'exploitation commerciale de ces espèces à Vanuatu et à d'autres endroits.

'MALTHUS' AND CHILLED FISH STORAGE

D. M. GIBSON

Torry Research Station, Aberdeen (United Kingdom)

Progress in developing rapid and automated methodologies in microbiology has been much slower and has occurred much later than in other life sciences such as biochemistry and clinical chemistry, but in recent years a number of approaches have been developed resulting in instruments which are available commercially. Examples are instruments which automate conventional procedures eg plating of samples, or improve upon microscopic examination eg direct epifluorescence of stained preparations, while other machines measure the activity of a culture eg by assay of the cellular ATP, or the heat production of a growing culture by microcalorimetry, or the release of $^{14}\text{CO}_2$ from radioactive labelled substrates. One of the most useful techniques is based on measurement of changes in the electrical properties of culture media during growth. As microbes grow, they metabolise the constituents of the medium and end products, usually of low molecular weight, accumulate. These compounds alter the electrical impedance of the culture. Instruments have been devised to measure these changes. One such instrument /1/, was devised at this Institute and the University of Aberdeen, and its use for measuring the quality of fish and predicting their shelf life at chill temperatures when spoilage is mainly by microbial activity is described here.

The instruments devised by Richards *et al.* for measurement of the changes in the AC conductivity of microbes growing in a nutrient medium have been called 'Malthus' (Malthus was the nineteenth century philosopher who described the mathematics of population growth and their consequences.) The instrument can handle up to 128 samples simultaneously. Essentially it presents the conductance component of the impedance at 10 kHz, freed from any contribution of capacitance by means of phase sensitive demodulation circuitry, in a digital form to a data logging-computer system which is used to calculate and display the change in conductance since inoculation of the sample. The samples, simple extracts in a nutrient medium, are placed in conventional 12 ml glass ampoules, which bear platinum electrodes, and are held in water baths whose temperature is controlled to $\pm 0.002^\circ\text{C}$ as the temperature coefficient of conductance is relatively high. A major advantage of using the instrument for routine microbiological testing is the speed of the response. The sensitivity of the measurement is such that the results are commonly available in a matter of hours, one fifth or less than the time taken in conventional assays, and those from highly contaminated samples even sooner, a difference not apparent by traditional techniques. The agreement between the data obtained by conventional assays and conductance measurements is good, especially with regard to the known errors of bacteriological counts. As well as determining the quality of a food at the time of sampling, there is considerable interest in knowing for how long the food will keep ie its rate of spoilage and the length of time before rejection. Because conventional microbiology is labour intensive and expensive in materials, the spoilage rate is usually determined at one temperature and the rates at other temperatures simply calculated using, for example, the Arrhenius equation. However, many microbiologists have been dissatisfied with the goodness of fit of their data in equations derived essentially for chemical reactions and recently Ratkowsky *et al.* /2/ have proposed another equation in which they relate the square root of the growth rate to absolute temperature

$$\sqrt{r} = b(T - T_0)$$

where r is the growth rate constant, T the temperature, T_0 a hypothetical temperature which is a property of the organism and b the regression coefficient. Based on an assessment of fish bacteriological data, in relation to this equation, Storey and Owen /3/ have proposed that the rates of spoilage of white fish from temperate waters at 5° and 10° are 2.25 and 4 times the rate at 0° , respectively. This paper tests their calculations and then shows how the shelf life of packaged fish can be predicted from Malthus conductance data.

2. EXPERIMENTAL

The data are taken from experiments on fish stored at chill temperatures. The data correlating bacteriological quality of fish with the results from Malthus assays are mainly given by Gibson *et al.* /4/ and those for shelf life studies by Cann *et al.* /5/.

3. RESULTS AND DISCUSSION

A typical plot of the conductance change (G_B) with sample incubation time is shown in Fig. 1. The shape of the curve closely resembles that of a bacterial growth

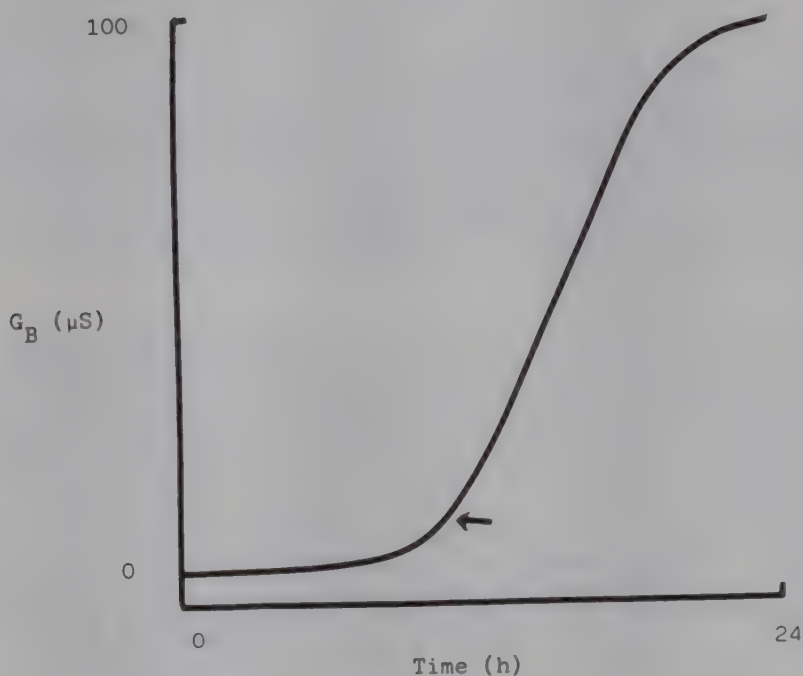


Fig. 1 - Change in conductance with time due to the growth of bacteria

curve, and indeed the relationship between change in conductance and change in number is very good /1/, being limited only by the errors in the bacterial count /6/ which greatly exceed those of the conductance measurements. The initial increase in G_B occurs when the count reaches ca 10^6 /ml. The time taken since inoculation for G_B to be significantly distinct from its starting value, is called the detection time (DT). Detection times correlate highly with the reciprocal of the logarithm of the microbial number thereby allowing Malthus data to be used instead of conventional counts. Separate calibration are required for each class of materials.

The reproducibility of the Malthus assay is high. For example, in a series of 12 replicate analyses of a herring extract, the mean detection time was 12.4 h and the standard deviation 0.7 h. Replicate conductance plots for pure cultures are virtually superimposable (see /7/) as are plots of the rates of change of conductance against G_B .

The conductance assays were done along side other quality measurements in a series of experiments on fish packaged under vacuum (VAC), or in modified atmosphere gas mixes (MAP) described by Cann *et al.* /5/. Fresh fish were packed and stored at 0, 5 or 10° until spoiled and the conventional bacteriological quality and eating quality indices were measured. The data were then analysed /6/ using the time-temperature relationship described by Ratkowsky *et al.* /2/ and Storey and Owen /3/. The arbitrary quality standard used was the taste panel score for cooked flavour (CF) /8/ and value of CF = 5.5 units was taken as the limit of shelf life. There was good agreement between the actual shelf life values found for the fish stored at 0°,

5° and 10° and the theoretical values calculated according to Storey and Owen from the 0° data all being within 0.5 d. At CF = 5.5 units, the mean bacterial count for all treatments was 3.1×10^7 /g fish tissue, and the Malthus detection time was 10 h. In addition, the correlation between taste panel score and detection times was high and linear over the whole time course of the experiment.

Accordingly, if a sample yielded a detection time greater than 10 h then it still possessed some shelf life which could be calculated as follows /6/. Because of the linear relationship between parameters mentioned earlier and storage time, it is possible to calculate, for each parameter at each temperature, its rate of change per day. Some examples are shown in Table I. Then, if a sample yielded a detection time of (P), greater than the rejection value (P_0), the remaining shelf life can be calculated by dividing the difference ($P - P_0$) by the appropriate daily spoilage rate anticipated from the expected storage temperature of the fish packs. Some examples are shown in Table II.

TABLE I

Daily spoilage rate for packaged fish

Storage Temperature (°C)	Cooked flavour (units/d)	Malthus Detection Time (h/d)
0	0.25	0.6
5	0.6	1.4
10	1.0	2.4

TABLE II

Some examples of calculated remaining shelf life

Let the cut off detection time $P_0 = 10$ h

Let the detection time found $P = 16$ h

Storage Temperature (°C)	Shelf life
0	$\frac{16 - 10}{0.6} = 10$ days
5	$\frac{16 - 10}{1.4} = 4.3$ days
10	$\frac{16 - 10}{2.4} = 2.5$ days

The user of such information can choose to use the shelf life pertaining to the worst storage conditions anticipated or the average expected storage temperature. The cut off quality standards chosen have been deliberately set better than the minimum limits of acceptance used by public health authorities so that there is some leeway for any errors accruing, without increasing the risks of offering for sale poor quality fish if the storage temperatures are higher than presumed, and helps to satisfy the desire to market good quality fish.

Malthus techniques, by their speed and automation, allowed for quality data to be available before chilled products are at the point of sale. In addition, they also speed up the detection of pathogens, or potential pathogens in fish. For

example, they have been used to detect Escherichia coli, coliforms and vibrios in fish /4/ and to detect Salmonella spp. in a variety of foods within one day /9/.

REFERENCES

1. J. C. S. RICHARDS, A. C. JASON, G. HOBBS, D. M. GIBSON and R. H. CHRISTIE : Electronic measurement of bacterial growth. J. Phys. E: Sci. Instrum. Vol.11 (1978) pp. 560-568.
2. D. A. RATKOWSKY, J. OLLEY, T. A. McMEEKIN and A. BALL : Relationship between temperatures and growth rate of bacterial cultures. J. Bact. Vol.149 (1982) pp. 1-5
3. R. M. STOREY and D. OWEN : A simple time-temperature function integrator. J. Fd Technol. in press.
4. D. M. GIBSON, I. D. OGDEN and G. HOBBS : Estimation of the bacteriological quality of fish by automated conductance measurements. Int. J. Fd Microbiol. Vol.1 (1984) pp. 127-134.
5. D. C. CANN, G. L. SMITH and N. C. HOUSTON : Further studies on marine fish stored under modified atmosphere packaging. Torry Research Station, Aberdeen (1983).
6. D. M. GIBSON : Predicting the shelf life of packaged fish from conductance measurements. J. appl. Bact. Vol.58 (1985) pp. 465-470.
7. A. C. JASON : A deterministic model for monophasic growth of batch culture of bacteria. Antonie van Leeuwenhoek. Vol.49 (1983) pp. 513-536.
8. J. M. SHEWAN, R. G. MacINTOSH, C. G. TUCKER and A. S. C. EHRENBURG : The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. J. Sci. Fd Agric. Vol.4 (1953) pp. 283-298.
9. M. C. EASTER and D. M. GIBSON : Rapid and automated detection of salmonellae by electrical measurements. J. Hyg. Vol.94 (1985) pp. 245-262.

LE "MALTHUS" ET L'ENTREPOSAGE DU POISSON REFRIGERE

RESUME : Les progrès récents de la méthodologie microbiologique permettent d'obtenir des résultats du contrôle de la qualité à temps pour les utiliser dans la détermination de la qualité avant que les aliments réfrigérés soient mis en vente. Ce rapport décrit un instrument, le "Malthus", et la façon de l'utiliser pour obtenir des résultats environ quatre fois plus vite qu'il n'est nécessaire habituellement pour les essais microbiologiques et aussi la façon d'utiliser les résultats dans un modèle de prévision de la durée résiduelle de conservation commerciale à toute température de réfrigération choisie.

CHEMICAL COMPOSITION AND QUALITY OF FROZEN ROLLER-PEELED KRILL MEAT

P. BYKOWSKI and W. KOŁODZIEJSKI
Sea Fisheries Institute, Gdynia, Poland

1. INTRODUCTION

Among food products obtained from Antarctic krill /*Euphausia superba* Dana/, shell-free muscles from the tail seem to be a high-quality product which could be successful on the world market.

At the Sea Fisheries Institute in Gdynia, a prototype production line was constructed; on the basis of original technology it allows for commercial-scale production of this product. Since the production of roller-peeled meat is on the increase, it is necessary to investigate the properties of the new product, especially its chemical composition, sensoric properties, shelf life during frozen storage, and other technological properties.

2. OBTAINING OF THE KRILL MEAT

The process of obtaining meat from krill consists of several operations including conditioning of the raw material, mechanical peeling on roller equipment, separation of meat from sea water, rinsing of the meat in fresh water, packaging, and freezing. Basically, the raw material for the production is fresh krill, processed immediately upon capture. The product made in this way is of a higher quality than that made from defrosted raw material. Up to 100 kg/h of the product are obtained from one peeler with the yield equalling 15-18% with respect to the weight of the raw material. The product obtained is in the form of meat mass consisting of individual muscles from krill tails. During contact freezing, it forms compact blocks which are final form of the product. Raw, frozen krill meat has to be defrosted and subjected to thermal treatment before consumption.

3. SENSORIC PROPERTIES OF THE PRODUCT

The appearance, colour, consistency, flavour, and taste of shell-free krill meat are typical for the meat of crustaceans, resembling the meat of small shrimp. Directly after peeling, the meat is

glassy, white-pink in colour. Individual muscles of the tails are firm, maintaining their natural, fibrous structure. The meat in its bulk is slightly sticky, which is caused by the dissolution of proteins during the production process. The flavour is not too intensive, pleasant, fresh, with a sea-water tang, the taste - sweet and salty, resembling the taste of shrimp meat. After thermal treatment, the meat is white-pink; its appearance resembles boiled rice. Its flavour is not too intensive with a discernible shellfish-like tang, the taste - sweet with a shrimp-like tang. The meat is firm, elastic, juicy, delicate in texture. Shell-free krill meat may be considered an analog of the meat of small shrimp, as far as its sensory properties are concerned.

4. CHEMICAL COMPOSITION

Basic chemical composition of raw, shell-free krill meat is the following: water - 82-85%, crude protein /Nx6,25/ - 12-13%, lipids - 0,7-1,1%, minerals - 1,3-1,9%, chlorides /NaCl/ - 1,1-1,7%, fluoride - 3,6-15 mg F/1000 g or 53-80 mg F/1000 g d.w., shell residue - 0,05-0,17%. A characteristic feature of roller-peeled krill meat is high water content, caused by high water holding capacity of krill proteins and the contact of the raw material with sea water during peeling and with fresh water during rinsing. For comparison, it may be said that the water content of krill tail meat obtained manually does not exceed 77-78%. Water absorbed during krill peeling is so strongly bound with proteins that it cannot be separated in a mechanical manner, e.g. by centrifuging. Part of the absorbed water is released from the meat during defrosting, resulting in large drip.

Thank to the fine separation of shell, containing over 90% of fluoride found in krill, the problem of krill toxicity as food has been solved. The meat contains 3-15 mg F/1000 g; the Food and Drug Administration of the USA has declared such amounts not harmful for human health. The FDA states that krill meat is considered as food and not only food additive /1/.

5. CHANGES DURING DEFROSTING

Two methods of defrosting have been used: - slow, at air temperature /13-20°C/, the time of defrosting a 1000 g block was about 16 hours, and - fast, in water, vacuum-packed blocks in polyethylene were placed in flowing water with a temperature of 12-15°C. Defrosting time in this case was about 4 hours. After defrosting the amount of free drip was determined, and then the amount of forced drip achieved by centrifuging the sample for 10 minutes with the

relative force of centrifuging of 1000 g. The chemical composition of the meat and free drip were also determined. The results are presented in Table 1 and Table 2.

TABLE 1

Amount of drip during defrosting of krill meat block
/% of the weight of frozen blocks/

Method and time of defrosting	Index ^{1/} n = 9	Drip		
		Free	Forced	Total
In air /16 h/	$\bar{x}$	33,7	17,7	51,4
	$E_{\bar{x}}$	$\pm 3,5$	$\pm 4,3$	$\pm 4,9$
In water /4 h/	$\bar{x}$	32,9	22,0	54,9
	$E_{\bar{x}}$	$\pm 2,9$	$\pm 1,3$	$\pm 2,0$

$\bar{x}$ - mean values from n = 9 samples
 $E_{\bar{x}} = s \frac{t}{\sqrt{n}}$ - boundary error of mean values where:
 s - standard deviation, n - number of measurements,
 t - coefficient of Student t - test for $\alpha = 0,05$

TABLE 2

Chemical composition of peeled krill meat and free drip
after defrosting

Method of defrosting	Sample	Index ^{1/} n = 9	Dry weight %	Proteins /Nx0,25/ %	$\frac{N_{22}}{1 \text{ tot.}}$	Ash %
In air /16 h/	Meat	$\bar{x}$	17,73	15,00	0,137	1,42
		$E_{\bar{x}}$	$\pm 0,48$	$\pm 0,05$	$\pm 0,015$	$\pm 0,13$
	Drip	$\bar{x}$	4,87	5,36	0,482	1,44
		$E_{\bar{x}}$	$\pm 0,21$	$\pm 0,29$	$\pm 0,038$	$\pm 0,20$
In water /4 h/	Meat	$\bar{x}$	18,36	15,34	0,133	1,74
		$E_{\bar{x}}$	$\pm 1,34$	$\pm 1,11$	$\pm 0,011$	$\pm 0,09$
	Drip	$\bar{x}$	4,78	5,02	0,500	1,34
		$E_{\bar{x}}$	$\pm 0,20$	$\pm 0,15$	$\pm 0,020$	$\pm 0,09$

$\bar{x}$, $E_{\bar{x}}$ as in Table 1

It was found that an average of about 33% of free drip is released during defrosting. No influence of the defrosting method on the amount of drip was observed. Since defrosted krill meat releases

large amounts of water, it contains more dry weight than frozen meat. Dry weight losses connected with drip are about 15% of its initial amount in the meat. The large amount of drip occurring during defrosting is a serious disadvantage of this product. It seems that amount of water remaining in the meat after defrosting at a level of 82% determines the level which should be achieved in the product before freezing in order to prevent excessive drip during defrosting.

6. THERMAL TREATMENT

Peeled krill meat should be subjected to thermal treatment prior to consumption. The investigations were aimed here at determining the optimum method and temperature range of the thermal treatment of krill meat. The main criteria were the sensoric properties of the meat and its physico-chemical changes resulting from heating. During first trials, it was found that the best method of thermal treatment is indirect heating in water bath with a temperature of about 75°C. It was also found that krill meat is suitable for consumption when heated to a temperature of 40-50°C. Heating it to a higher temperature results in deterioration of all sensoric properties, especially taste, flavour, and texture.

Table 3 presents total amounts of drip taking place during defrosting and thermal treatment of krill meat. Chemical composition of meat and drip after defrosting and after thermal treatment are presented in Table 4.

TABLE 3

Drip losses after thawing and heating of the peeled krill meat
/as percent of the frozen meat weight/

	Heating temperatures /30 min/			
	+40°C	+50°C	+60°C	+70°C
Free thawing drip	29,5	29,5	29,5	29,5
Free thermal drip	23,2	26,1	28,3	32,7
Total drip	52,7	55,8	57,8	62,2
Yield of the meat	47,3	44,2	42,2	37,8

TABLE 4

Chemical composition of peeled krill meat and drip
after thawing and heating /30 min/ at various temperatures

Process		Sample	Dry weight %	Protein /Nx6,25/ %	$\frac{N_{np}}{N_{tot.}}$
Thawing		Meat	19,4	16,2	0,20
		Drip	6,7	4,6	0,55
Heating at temperatures	+40°C	Meat	24,2	20,4	0,14
		Drip	7,7	5,8	0,52
	+50°C	Meat	25,5	21,8	0,13
		Drip	7,4	5,6	0,53
	+60°C	Meat	27,5	23,7	0,11
		Drip	7,4	5,4	0,54
	+70°C	Meat	28,3	24,6	0,10
		Drip	6,8	5,0	0,55

7'. FROZEN STORAGE SHELF LIFE

Shelf life in frozen storage was investigated for two kinds of meat - rinsed and not rinsed in fresh water after peeling. Meat samples were frozen in a plate freezer, in the form of standard blocks in boxes of waxed cardboard, and then stored at a temperature of -25°C.

Sensoric changes during storage were estimated by a test panel evaluation using a five-grade hedonic scale; 5 points - very good, 4 - good, 3 - acceptable, 2 - not acceptable, 1 - bad. Meat samples were prepared for investigations by indirect heating in water bath with a temperature of 65-70°C until the meat had a temperature of +45°C; the drip was then separated.

The appearance, flavour, taste, and texture of meat after thermal treatment were evaluated; on this basis, the general quality of the meat was determined on a five-grade scale. The results of sensoric evaluations served to determine a linear regression of the dependence of the general quality of peeled krill meat on the frozen storage time at a temperature of -25°C, Fig. 1.

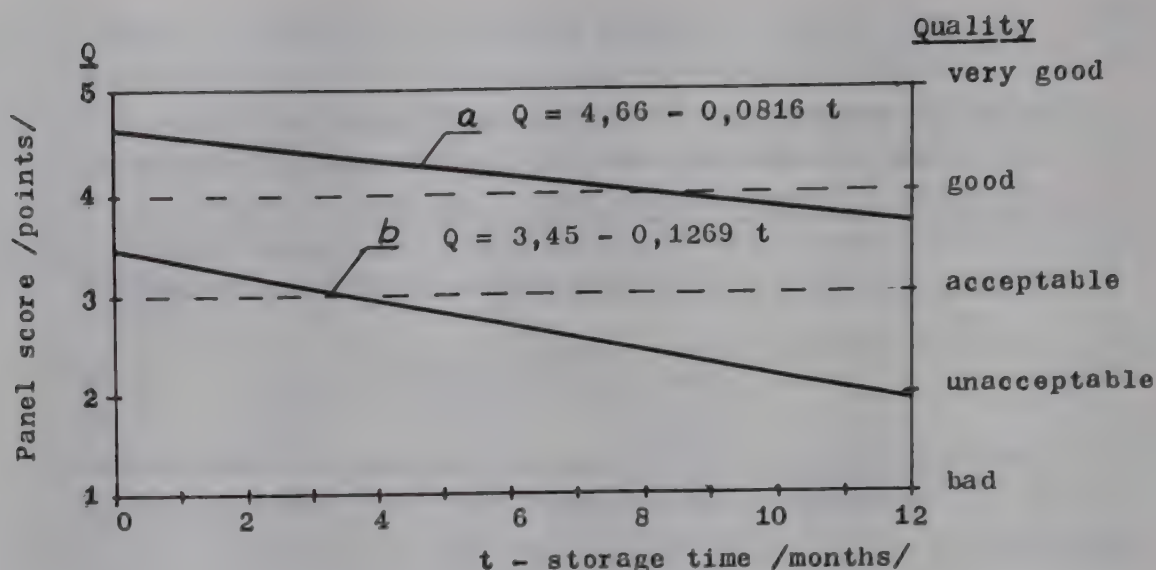


Fig. 2 Frozen storage shelf life of krill meat
a - rinsed, b - not rinsed in fresh water after peeling

The quality of krill meat rinsed in fresh water was better than good /4,7 pts. after peeling/. This meat was stable during frozen storage. After 12 months of storage at a temperature of -25°C , its quality was still close to good /3,7 pts./.

8. CONCLUSIONS

1. The use of roller peelers and proper technology allows for the production of pure krill meat on a commercial scale and its use as food for human.
2. The sensoric properties of peeled krill meat are typical for crustacean meat. As far as taste, flavour, and texture are concerned, it resembles the meat of small shrimp and may be used as its analog in the food industry.
3. Meat produced from fresh krill on board fishing vessels maintains good quality for 12 months, when stored at a temperature of -25°C .

REFERENCES

1. Anon.: FDA says krill is a food, not food additive.
Food Chemical News, July 6, p. 45 /1983/

COMPOSITION CHIMIQUE ET QUALITE DE CHAIR DE KRILL DECORTIQUEE AU ROULEAU

RESUME : Les auteurs ont mis au point une technique pour obtenir la chair de krill par la méthode du rouleau. La chair de krill frais obtenue de cette façon est brillante, rose pâle et a une odeur agréable et un goût doux de crustacé. Sa composition chimique de base est la suivante : 82 à 85 % d'eau, 12 à 13 % de protéine brute (Nx6,25), 0,7 à 1,1 % de lipides, 1,3 à 1,9 % de cendres, 1,1 à 1,7 % de NaCl. La chair contient des restes de carapace (environ 0,1 %) avec une concentration en fluor de 8 à 13 ppm. Ce niveau de fluor ne constitue pas un danger pour la santé humaine.

Les modifications de la qualité de la chair décongelée, provoquées par le traitement thermique entre 40 et 80°C, ont été déterminées. On a trouvé que la meilleure chair pour la consommation (présentant les meilleures qualités organoleptiques) était obtenue par chauffage à une température de 40 à 50°C. Le rendement de la chair après un tel traitement est d'environ 50 % en masse de chair congelée. Des températures plus élevées provoquent une détérioration de la qualité du produit et du rendement.

Des recherches sur la conservation de détail du produit ont montré que la chair rincée à l'eau douce après la préparation conservait une bonne qualité pendant 12 mois lorsqu'elle était entreposée à une température de -25°C. La qualité générale de la chair non rincée s'abaisse au-dessous du niveau acceptable dès 3 mois en raison du goût amer croissant.

On a trouvé que du point de vue de la qualité, la chair de krill décortiquée au rouleau était une bonne matière première pour la fabrication de produits analogues aux produits à base de crevette, y compris les produits appertisés.

DISCUSSION

W. SCHREIBER (FRG) - When you investigated the shelf content of krill, which reference base did you use ?

P.J. BYKOWSKI - We determine shell content immediately after peeling by alkali hydrolysis at about 90°C, washing with water to neutralise, then drying to stable weight - that is immediately after peeling. The shell removed is 0.05-0.17% of the product.

CHANGES IN PROTEIN FUNCTIONALITY IN FISH MUSCLE

F. JIMENEZ-COLMENERO, M. TEJADA, and A.J. BORDERIAS
Instituto del Frío, Ciudad Universitaria, 28040 Madrid (Spain)

1. INTRODUCTION

Several functional properties (protein solubility, emulsifying capacity, and apparent viscosity) of fish muscle proteins from different species were studied in relation to protein concentration in the various protein fractions and to frozen storage (-12 and -20 °C) in an effort to determine the behaviour of frozen muscle and to develop a simple quality control method. The influence of other factors was also examined.

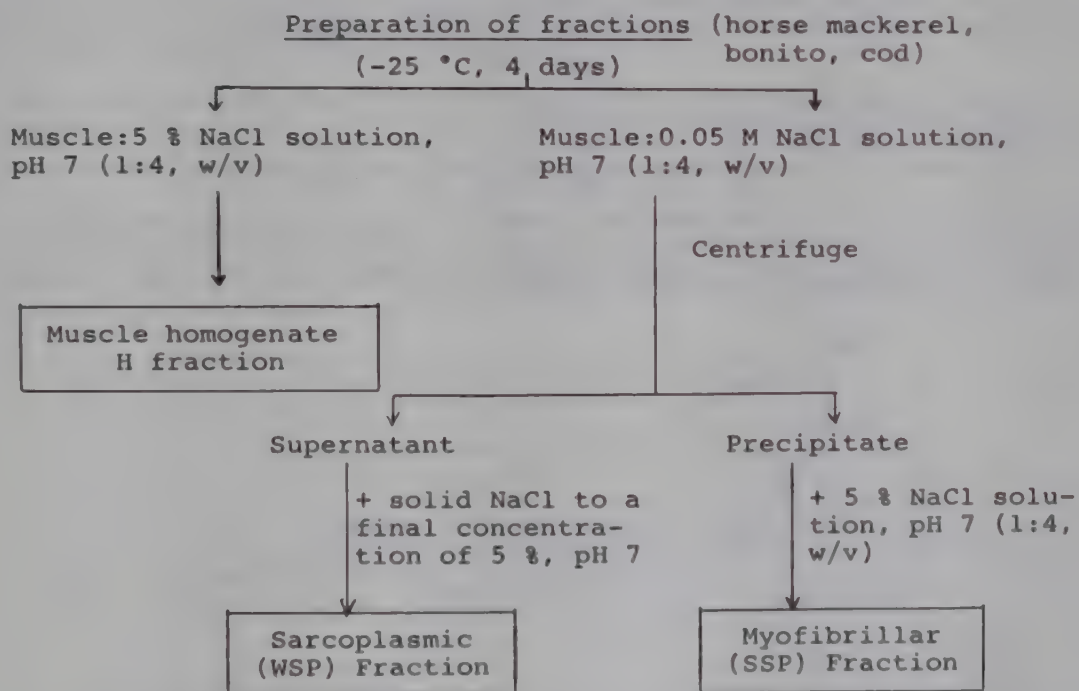
2. MATERIALS AND METHODS

Horse mackerel (*Trachurus trachurus* L.), blue whiting (*Micromesistius poutassou* Risso), bonito (*Sarda sarda* Bloch), and cod (*Gadus morhua*, L.) were employed. Samples were prepared by mincing the muscle in a conventional mincer using an orifice diameter of 5 mm. They were then frozen, vacuum-packed, and treated for study as indicated in Fig. 1.

Minced fish muscle

Frozen storage

- 20 °C*, 8 months (horse mackerel, blue whiting)
- 12 °C, 7 months (horse mackerel, bonito, cod)



The samples were subjected to temperature variation once during the first month of storage

Fig. 1. Temperature, storage time, treatment, and preparation of fractions for the species studied

The functional properties studied were the percent protein solubility in 5 % NaCl (PS) /1/; emulsifying capacity (EC) (g of oil/20 g of protein extract) /2/; and apparent viscosity (η_{app}) expressed in centipoises (cP) /3/. The amount of protein present in the different fractions was obtained by Kjeldahl's method with a factor of 6.25.

The degree of significance of the correlations was taken from Lamotte's tables /4/.

3. RESULTS AND DISCUSSION

The reduction in the PS and EC with storage time (Figs. 2 and 3) was more marked in the gadids than in the other species. The initial decrease in protein solubility and emulsifying capacity in the two species stored at -20°C appeared to be due to the temperature increase to which these lots were subjected (to between -6 and -10°C) during the first month of storage. The changes resulting from this temperature variation led to a larger decrease in these functional properties than that occurring during the entire storage period (7 months) of the other lot (stored at -12°C). The most pronounced reduction in these two parameters in the conditions tested took place in cod.

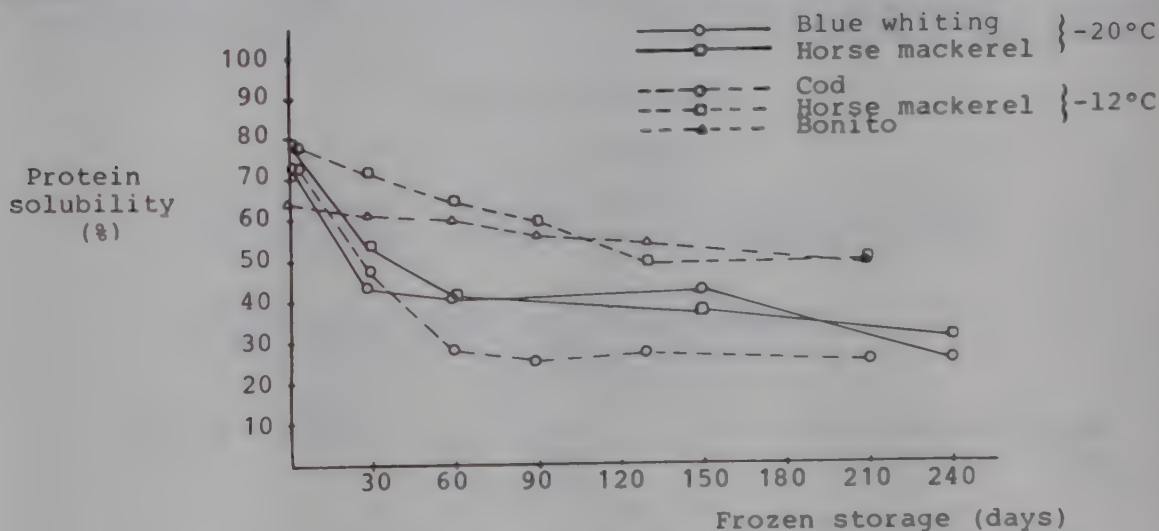


Fig. 2. Changes in protein solubility (PS) with storage time

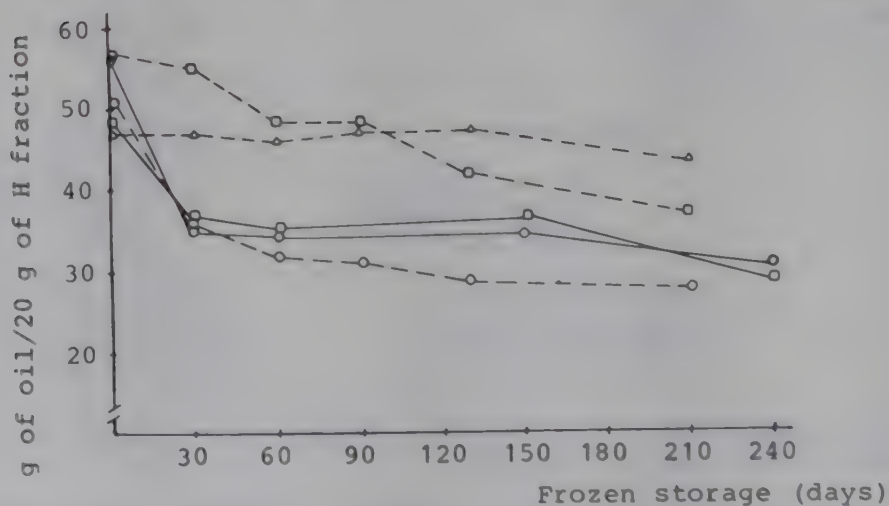


Fig. 3. Changes in emulsifying capacity (EC) with storage time

Initially apparent viscosity (Fig. 4) was greater in the gadids than in the other species, though it decreased at a more rapid rate during frozen storage. This high initial viscosity may be due, among other causes, to the fact that the effect of pH was greater in the cod (6.89), blue whiting (6.96), and horse mackerel stored at -20°C (6.29) than in the bonito (5.77) and horse mackerel stored at -12°C (5.90). As storage progressed the reduction in protein solubility brought about a decrease in η_{app} , so the effect of pH could no longer be assessed.

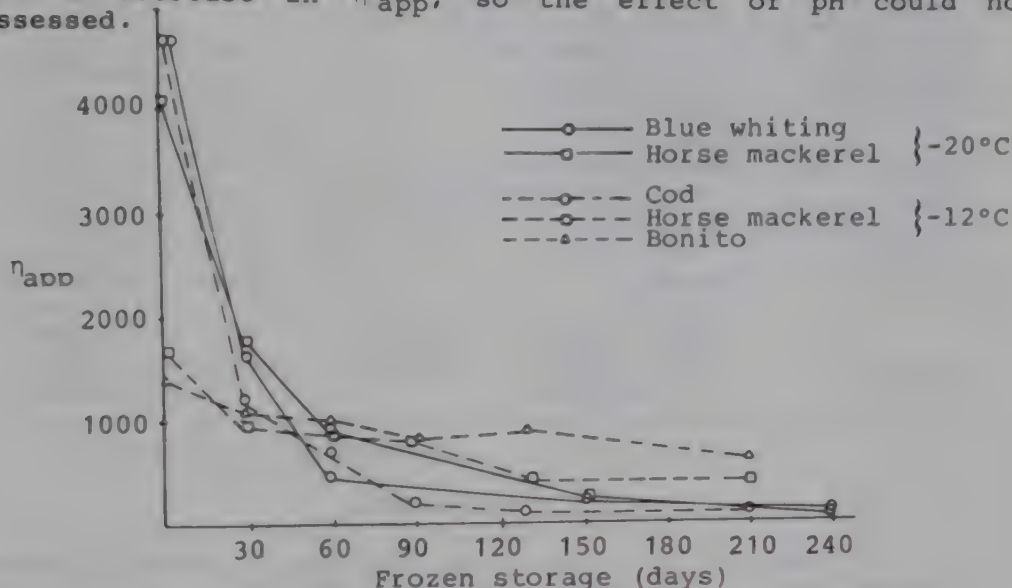


Fig. 4. Changes in apparent viscosity (η_{app}) with storage time

The larger drop in η_{app} in horse mackerel stored at -20°C compared to that stored at -12°C can be ascribed to the previously mentioned temperature increase (to between -6 and -10°C), which also contributed to the decrease in PS and EC.

The viscosity in the muscle homogenates was primarily a factor of the myofibrillar protein, since the WSP fraction did not register on the measurement scale under the conditions of this experiment /3/.

A linear relationship was found between EC and soluble protein concentration in each of the three fractions (H, SSP, and WSP), independent of the species (bonito, cod, and horse mackerel) (Fig. 5).

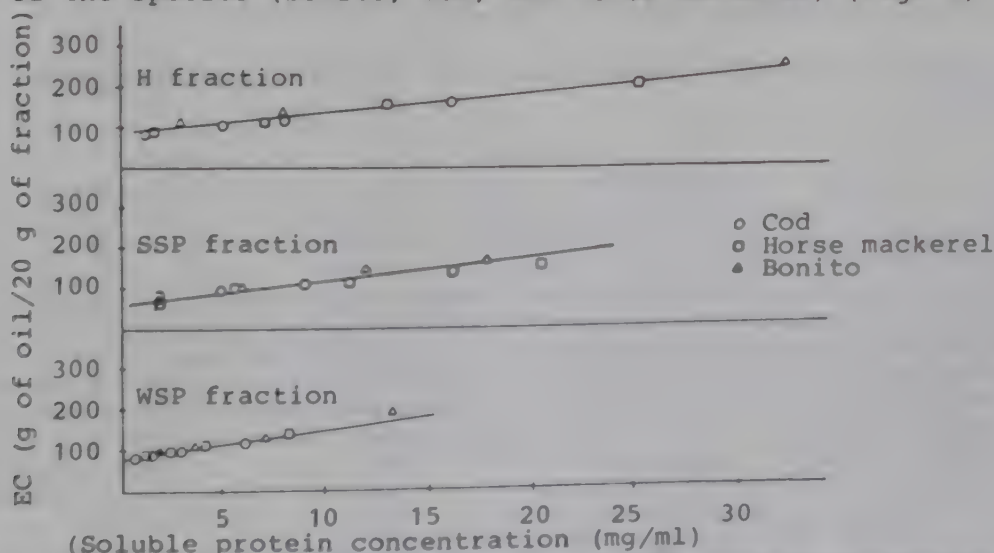


Fig. 5. Emulsifying capacity (EC) on soluble protein concentration (mg/ml) for the different fractions

A high correlation ($P < 0.01$) was found to exist between protein solubility, emulsifying capacity, and apparent viscosity in all the species studied (Table I).

TABLE I

Correlation coefficients between the functional properties studied ($P < 0.01$)

	Species	EC	η_{app}
PS	Bonito	0.87	0.91
	Cod	0.95	0.97
	Horse mackerel (-12 °C)	0.77	0.95
	Horse mackerel (-20 °C)	0.88	0.98
	Blue whiting	0.91	0.92
EC	Bonito		0.82
	Cod		0.98
	Horse mackerel (-12 °C)		0.89
	Horse mackerel (-20 °C)		0.92
	Blue whiting		0.95

REFERENCES

1. J.I.M. IRONSIDE and R.M. LOVE, Studies on protein denaturation in frozen fish I. Biological factors influencing the amounts of soluble and insoluble protein present in the muscle of the North Sea cod. *J. Sci. Food Agric.* (1958) pp. 597-617.
2. F. JIMENEZ-COLMENERO and E. GARCIA-MATAMOROS, Effects of washing on the properties of mechanically deboned meat. Proceedings of the 27th European Meeting of Meat Research Workers (O. Prandl, ed.), Vienna (1981) pp. 352-354.
3. A.J. BORDERIAS, F. JIMENEZ-COLMENERO, and M. TEJADA, Viscosity and emulsifying ability of fish and chicken muscle protein. *J. Food Technology* 20 (1985) pp. 31-42.
4. M. LAMOTTE, Estadística biológica (Biological Statistics). S.A. Toray-Masson Ed., Barcelona, Spain (1981) p. 159.

MODIFICATIONS DES PROPRIETES FONCTIONNELLES DES PROTEINES DU TISSU MUSCULAIRE DE POISSON

RESUME: On étudie diverses propriétés fonctionnelles (solubilité des protéines, émulsibilité et viscosité) des protéines du tissu musculaire de plusieurs espèces de poisson (chinchard, merlan bleu, cabillaud et bonite) en fonction de la concentration en protéines des différentes fractions protéiques (totale, sarcoplasmique et myofibrillaire) pendant la conservation à l'état congelé à -12 et -20°C. On examine également les effets d'autres facteurs. Il est constaté qu'au cours de l'entreposage, les propriétés fonctionnelles en question diminuent plus vite chez les gadidés que chez les autres espèces. L'émulsibilité (g d'huile/20 g de tissu musculaire homogénéisé) est fonction de la concentration en protéine soluble des différentes fractions et non de l'espèce. La viscosité apparente initiale est plus grande chez les gadidés que chez les autres espèces. Elle baisse néanmoins plus rapidement pendant la conservation à l'état congelé. La viscosité du tissu musculaire homogénéisé dépend essentiellement des protéines myofibrillaires, encore qu'il existe d'autres facteurs augmentant la viscosité totale. Dans toutes les espèces, on relève une nette corrélation entre les différentes propriétés fonctionnelles étudiées ($P < 0,01$).

P. Nesvadba
Ministry of Agriculture, Fisheries and Food, Torry Research Station,
135 Abbey Road, Aberdeen (United Kingdom)

1. INTRODUCTION

Chilled and frozen products in open refrigerated retail display cabinets are subjected to a considerable heat flow input from the warmer shop. The heat reaches the products because the cabinets are open to the shop, a design feature that is undesirable from the physics/engineering point of view but demanded by the established retail trade practices. Although there are some signs of an increasing use of closed display cabinets, particularly in the quality food stores, the widespread use of the open cabinets is likely to persist for the foreseeable future. It is therefore important to understand and quantify the nature of heat transfer to the products and to take measures to minimize the detrimental effect on the storage life of the products.

In particular, it is necessary to estimate the magnitudes of the four possible components of the heat input

- C1: black body radiation from walls, ceiling and other warm surfaces in the shop
- C2: air convection (draughts or turbulence caused by forced circulation)
- C3: condensation (in the form of frost) of ambient water vapour
- C4: conduction through air.

In addition it is necessary to measure the total emissivity of foods or food packages at the display temperatures and to assess the effect of the increased top layer temperature on the storage life of the products.

A number of studies of heat transfer into cabinets have already been made /1-5/. The purpose of this paper is to present a method for the measurement of the components C1 and C2 + C3 of the heat input, a method for the measurement of the total emissivity of chilled and frozen foods and an estimate of the effects of the heat input on the storage time of products.

2. ESTIMATION OF THE COMPONENTS OF THE HEAT INPUT

Theoretical considerations

Fig. 1 shows a schematic drawing of the heat flows in a retail cabinet. An estimate of the radiative heat flux, q_1 , can be obtained from

$$q_1 = \epsilon \sigma F (T_a^4 - T_t^4) = \epsilon q_0 \quad (1)$$

In deriving eq.(1) it was assumed that the top surface of the products is not concave (cannot "see" itself) and that the shop is much larger than the cabinet - then the shop can be regarded as a hohlraum, that is, as a black body, and the emissive properties of the shop ceiling, walls etc play no role at all /6/. This fact is not always recognised in the literature /1,3/. For typical conditions in a cabinet $\epsilon = 0.9$, $T_a = 293 \text{ K}$, $T_t = 261 \text{ K}$ and the radiative heat flux reaching the top surface is 141 W m^{-2} .

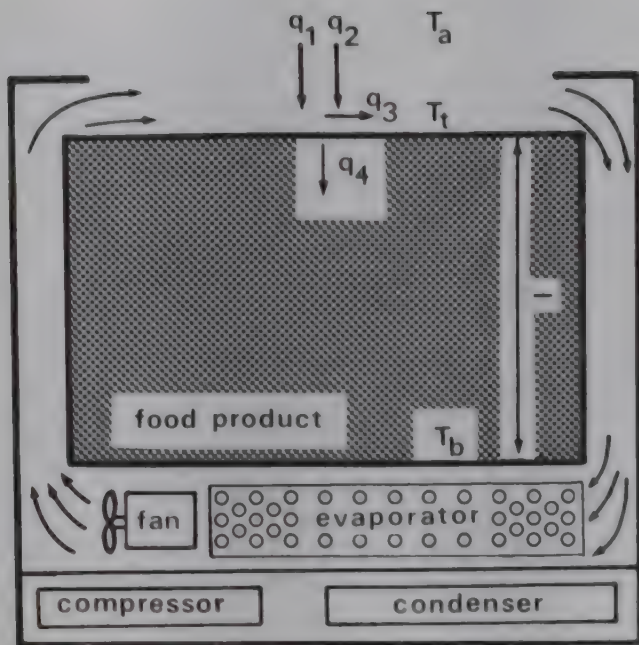


Fig. 1 - Schematic diagram of heat flow in a cabinet.

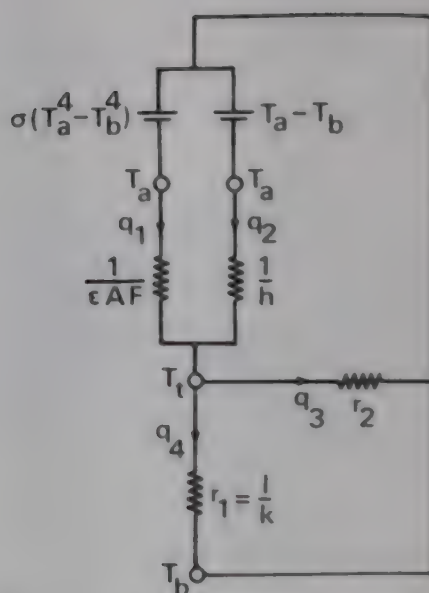


Fig. 2 - Electrical model of heat flow in a cabinet.

The magnitude of the air convection/infiltration heat flux can, in principle, be estimated from

$$q_2 = h (T_a - T_t) \quad (2)$$

However, the value of h is difficult to estimate theoretically and can range from 5 to $10 \text{ W m}^{-2} \text{ K}^{-1}$ depending on the pattern of air movement at the top layer [3]. Thus q_2 can range from 160 to 320 W m^{-2} .

It can be shown by calculation that conduction through ambient air is normally a negligible part of the total heat flux. Likewise, in a properly designed cabinet the ambient moisture condenses onto the evaporator leaving the top surface frost free (except possibly during the defrost cycle when temperature fluctuations occur). Thus the radiation and convection (q_1 , q_2 in Fig. 1 and Fig. 2) are the main components of the total heat flux incident on the product surface. A part of the incident flux is absorbed by the cooling air (flux q_3 in Fig. 1 & 2), the remaining flux q_4 flowing into the product. If q_4 and the product thermal resistance, r_1 , are known, then the temperature T_t can be calculated using the electrical network model of heat flow (Fig. 2).

Experimental

The heat fluxes q_1 , q_4 and the difference $q_2 - q_3$ were determined using four heat flow sensors in good thermal contact with a metal plate whose temperature was measured (T_t). The plate was positioned at the load line of a cabinet. The exposed surfaces of the sensors were treated by coating with aluminium foil and matt black paint in order to achieve different emissivities of the sensors. These were $\epsilon = 0.10, 0.68, 0.81$ and 0.95 , determined by the radiometric method described in section 3.

The heat flux through the sensors

$$q_4 = q_2 - q_3 + q_1 = q_2 - q_3 + \epsilon q_0 \quad (3)$$

varies with the sensor emissivity, ϵ . By plotting a graph of q_4 versus ϵ (Fig. 3) and extrapolating to $\epsilon = 0$, the intercept $q_4(0)$ is obtained which represents the

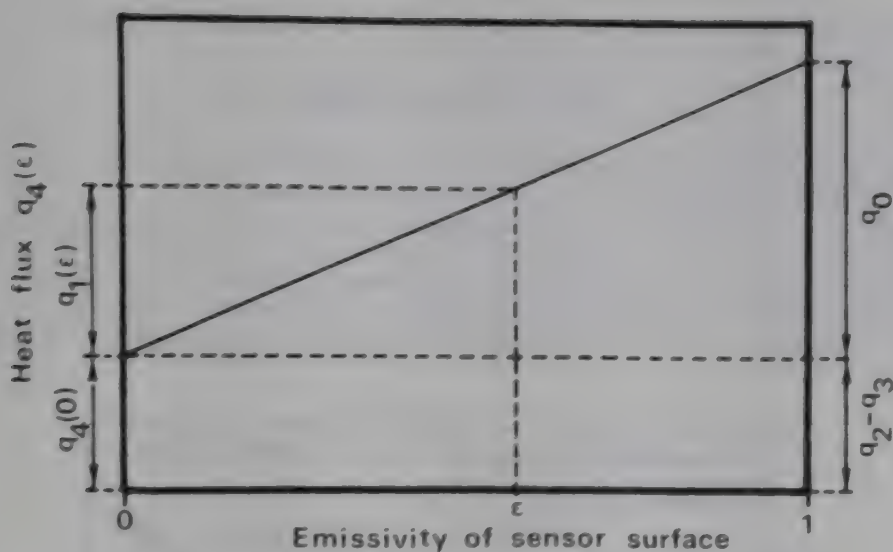


Fig. 3 - Principle of the method of measuring heat fluxes.

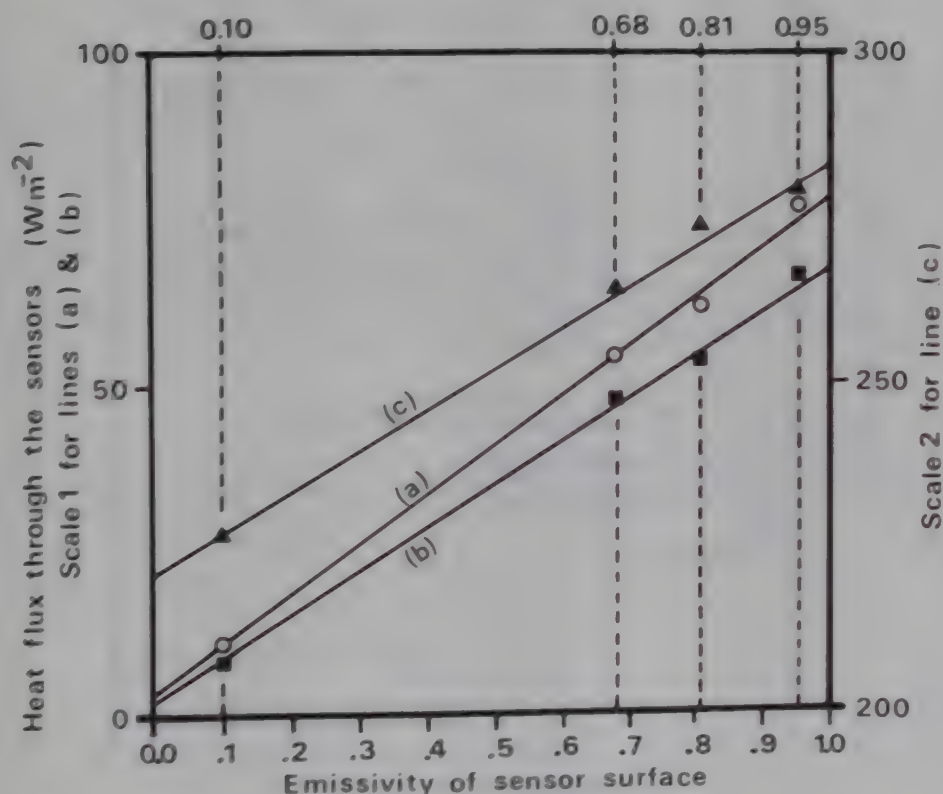


Fig. 4 - Results of heat flux measurements.

heat flux into the product caused by ambient air convection and conduction. Also, for any product emissivity, the values of q_1 and q_4 can be obtained from the graph.

Fig. 4 shows the results of heat flux measurements for three situations where the heat flows in a conduction type cabinet ($q_2 = 0$) were simulated in the laboratory.

For an absorbing surface at $T_p = -18^\circ\text{C}$, with $T_a = 25^\circ\text{C}$ and natural convection

(lines (a) and (b)), q_1 amounts to most of the total net flux. The use of an aluminium foil reflector 55 x 122 cm positioned 1 m above the sensors reduced q_1 by about 1% (line (b), scale 1). The effect of forced air convection and infiltration was simulated by blowing ambient air across the top of the cabinet at 0.5 ms^{-1} , $T_p = -12^\circ\text{C}$, $T_a = 25^\circ\text{C}$. This increased the heat flux considerably (line (c), scale 2).

It is hoped to use this method in an actual supermarket situation and to estimate the thermal resistance of product stacks.

3. EMISSIVITY MEASUREMENTS

To assess the heat input by radiation, the emissivities of various foods and packaging materials were measured using the heated radiometer [7] and a refrigerated sample holder enabling measurements to be made at -18°C . The radiometer, maintained at 98°C loses heat to the sample and generates a voltage proportional to the heat flow and related to the emissivity of the sample. Three materials with known emissivities (polished aluminium, abraded stainless steel and black paint) were used to obtain calibration curves for the radiometer voltage (open circles in Fig. 5).

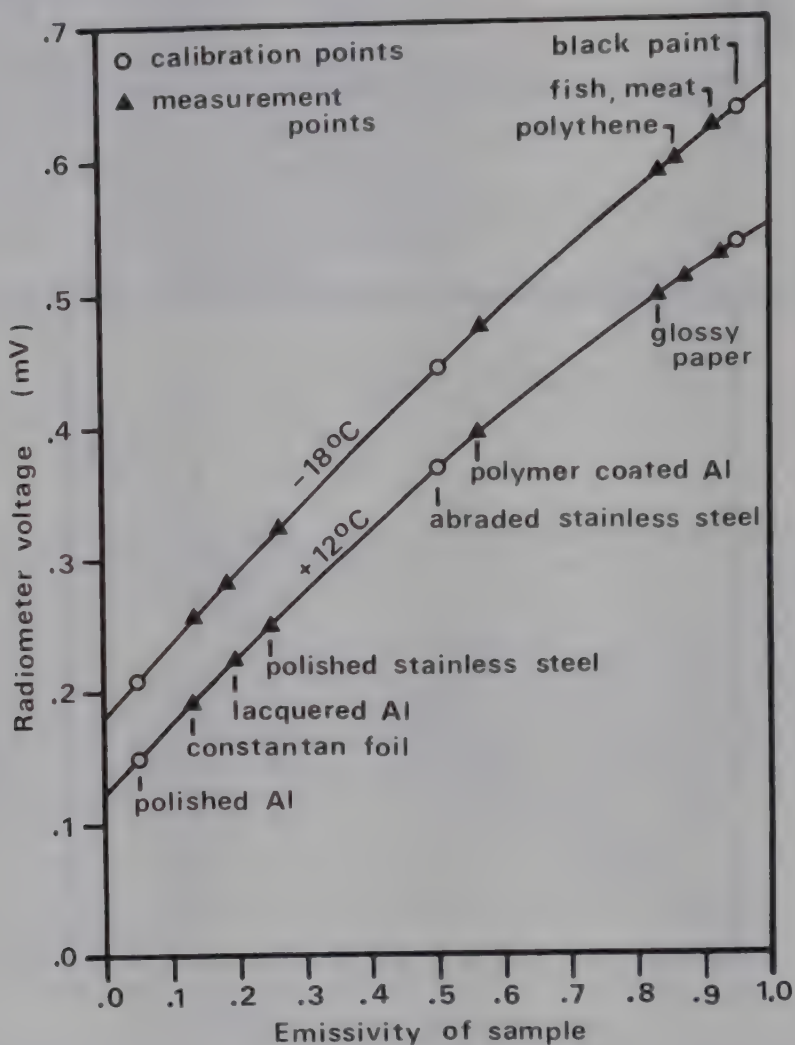


Fig. 5 - Results of emissivity measurements.

In Fig. 5 the results of measurements at -18°C are compared with data obtained at 12°C . It can be seen that the emissivities do not depend greatly on temperature. The emissivity of fish is practically unchanged on freezing, the emissivity of ice being only slightly smaller than that of water.

The commonly used packaging materials have high emissivities. The use of some form of metallisation could reduce these to 0.2-0.3 (lacquered aluminium).

4. STORAGE TIME IN THE TOP LAYER OF OPEN CABINETS

Recent /8/ and earlier /2/ surveys of supermarket cabinets revealed that more than 37% had T_t greater than -12°C , the maximum recommended by British Standard 3053. It is of interest to estimate the equivalent storage times X_t and X_b in the top layer at $T_t = -12^\circ\text{C}$ and the bottom layer at $T_b = -18^\circ\text{C}$ respectively, after which the product suffers the same quality deterioration.

Whilst the quality-time-temperature relationships are well established for unfrozen foodstuffs, for the frozen foodstuffs they are still at the development stage. However, taking for illustration the equation for the quality index given in reference /9/,

$$Q = 100 - a \exp \left(-\frac{B}{RT} - \frac{C}{X} \right) \quad (4)$$

the storage time in the top layer is

$$X_t = \left[\frac{1}{X_b} + \frac{B}{CR} \left(\frac{1}{T_b} - \frac{1}{T_t} \right) \right]^{-1} \quad (5)$$

The values of B and C depend on the type of foodstuff and on the attribute taken as the measure of quality. With $B = 16.4 \text{ kJ mol}^{-1}$ and $C = 6.2 \times 10^{-3} \text{ day}^{-1}$ for the texture score of frozen fish /9/ and taking the mean storage time in the deep layer to be $X_b = 15$ days /8/, the equivalent storage time in the top layer is $X_t = 10.1$ days, that is, 5 days shorter. This reduction is probably an underestimate since eq.(5) does not take into account the increase in the rate of deterioration of foodstuffs due to temperature fluctuations at defrost cycles. On the other hand, it should be noted that in practice the reduction in storage time is offset by the reduction in the residence time of the product in the top layer (6 days shorter median time in the top layer compared to the bottom layer /8/).

5. CONCLUSIONS

The surfaces of chilled and frozen products have high emissivities and radiation is a significant component of the heat flux incident on the top layer of products. The top layer temperature of -12°C causes an estimated 5 days reduction in the storage time of frozen fish.

LIST OF SYMBOLS

a	dimensionless constant
A (m^2)	area of the product exposed to the shop or the area of the service opening
B (J mol^{-1})	activation energy for spoilage process
C (day^{-1})	rate of spoilage
F	view factor for radiation exchange between the product (or the sensor) and the shop (or laboratory)
h ($\text{W m}^{-2} \text{ K}^{-1}$)	average convection heat transfer coefficient
k ($\text{W m}^{-1} \text{ K}^{-1}$)	effective thermal conductivity (product + package)
l (m)	depth of product stack
q_o (W m^{-2})	maximum possible radiation heat flux into the cabinet (for $\epsilon = 1$)
q_1 (W m^{-2})	radiation heat flux into the cabinet
q_2 (W m^{-2})	air convection/infiltration heat flux into the cabinet

q_3 ($W\ m^{-2}$)	heat flux removed from the top surface by cooling air
q_4 ($W\ m^{-2}$)	net heat flux into the top surface of the product stack
Q	centesimal quality index
r_1 ($KW^{-1}\ m^2$)	thermal resistance of product stack per $1\ m^2$ of area ($r = 1/k$)
r_2 ($KW^{-1}\ m^2$)	thermal resistance for heat removal by cooling air per $1\ m^2$ of product area
R	$8.31\ J\ mol^{-1}\ K^{-1}$, the universal gas constant
T (deg)	temperature of product, general
T_a (deg)	temperature, ambient
T_b (deg)	temperature at the bottom of product stack
T_t (deg)	temperature at the top of product stack
X_b (day)	storage time at temperature T_b
X_t (day)	storage time at temperature T_t
ϵ	total emissivity (hemispherical) of the product (or sensor) surface
σ	$5.67 \times 10^{-8}\ W\ m^{-2}\ K^{-4}$, the Stefan-Boltzmann constant

REFERENCES

1. A.E. HAWKINS, C.A. PEARSON and D. RAYNOR : Advantages of low emissivity materials to products in commercial refrigerated open display cabinets. Proc. Inst. Refrig. 69 (Session 1972-73) pp 54-64.
2. G. LORENTZEN and R.S. HEIERSTED : Air infiltration in open freezer cabinets. Kältetechnik-Klimatisierung 21 (1961) pp 317-323.
3. D. BARRILLON, R. BARTOLI and A. GAC : Optical qualities of frozen food packs (in French). Revue Générale du Froid, No. 5 (1971) pp 419-426.
4. R. MALTON : Observations on refrigerated retail display cabinets in the UK. Proc. MRI Symp. "Meat chilling - why and how?" Meat Research Institute, Langford, Bristol, ed. C.L. Cutting (1974) pp 29.1-29.11.
5. C. BAILEY : The performance of refrigerated meat display counters. Refrigeration and Air Conditioning (1972) pp 35-42.
6. D.K. EDWARDS : Radiation heat transfer notes. Hemisphere publ. corp., Washington, New York, London (1981) pp 124-125.
7. P. NESVADBA : Measurements of total emissivity of chilled and frozen foods. High Temp.-High Pressures (1985) to be published.
8. J.W. WIGNALL and J.R. BURT : Frozen Fish Distribution in the United Kingdom; Torry Research Station, Aberdeen, unpublished data.
9. J. MORENO : Quality deterioration of refrigerated foods and its time-temperature mathematical relationships. Int. J. Refrigeration 7 (No. 6) 1984, pp 371-375.

TRANSFERT DE CHALEUR PAR RAYONNEMENT AUX PRODUITS DANS LES MEUBLES DE VENTE FRIGORIFIQUES

RESUME : Des produits réfrigérés congelés, en meubles de vente ouverts, reçoivent par les ouvertures, une quantité de chaleur considérable provenant du magasin qui est plus chaud. La couche supérieure des produits est en conséquence plus chaude que les couches sous-jacentes, ce qui est préjudiciable à la qualité des produits. Ce rapport présente des techniques de mesure de l'apport de chaleur et de l'émissivité des produits. Il est fait une estimation de la réduction du temps de conservation des produits de la couche supérieure.

DEVELOPMENT AND USE OF A TASTE PANEL FOR THE DETERMINATION OF SHELF LIFE

D.N. SCOTT
M.G. HOGG

Division of Horticulture and Processing,
Department of Scientific and Industrial Research (New Zealand)

Sensory evaluation has been recognised for some time as an important procedure to complement alternative methods of fish quality evaluation - namely biochemical and microbiological procedures. In the determination of shelf-life of a fish product it is implicit that at some stage the product has been assessed by a taste panel.

The majority of reported fish research using taste panels has reported drawing members of the panel from within the research establishment - a procedure that is quite unsatisfactory if there is considerable time involved. Also there may be bias due to prior knowledge about the experiment.

The aim of this current work was to develop a taste panel for research on the shelf life of fish. The authors sought to establish a panel that would not be restricted by the time involved, that would not be biased by prior knowledge but would be highly motivated and confident and able to assess a wide variety of fish and fish products. Sensory profiling using descriptive terms to describe the odour, texture and flavour was the selected sensory technique.

1. DEVELOPMENT OF THE TASTE PANEL

Selection of Personnel

The recruitment of personnel was undertaken after publishing articles in several local newspapers. These articles described the general sensory evaluation work being carried out at the research centre on fish and horticultural products. Approximately 200 local people expressed an interest in becoming involved. A questionnaire was sent to each of them in which information was sought about general health, special food likes and dislikes and more specifically, the frequency of consumption of a variety of fish and horticultural crops. About 80 of the volunteers, based on their having indicated that they were reasonably frequent consumers of seafood were approached, and invited to take part in some fish tasting work.

The 80 volunteers were subjected to a screening programme in which each participant made 3 visits to the research centre to complete 8 triangle tests involving fish of different species and freshness. During the third visit, informal round-table discussion took place and the ability and willingness of each person to participate within the group was assessed. Twenty-seven people were then chosen for further involvement. The choice of these people was based on their sensory acuity with respect to fish, their demonstrated effective written and verbal communication skills, and their having shown a high level of motivation about the general research programme.

Establishment of the Sensory Evaluation Team on Fish (SETOF)

Those 27 people who had been selected in the screening programme, were approached concerning the formation of SETOF (The name given to the "Sensory Evaluation Team on Fish"). Contact was made via a letter. In this, the importance of sensory evaluation to the general fish research programme was outlined and the need for a group of people from outside the research centre who could objectively assess fish quality was explained. The point was made that in the previous 3 fish tasting sessions their efforts had been noted positively, and so they were now being invited to attend a seminar at which their potential role as a member of the research group would be discussed. The seminar re-iterated these points and the time involvement for both the orientation programme and longer term research projects.

Orientation Programme

Volunteers attended a 17 week orientation programme, which involved 14 training sessions and 3 social events. Full details of the orientation programme have been described elsewhere /1/. Each session was repeated on 4 occasions over one week. This allowed each person to select the time most convenient for him/her. A maximum of 6 people attended any one session and every attempt was made to mix individuals within the groups. Typically a session lasted 30-45 minutes and took the following format:

- A. While everyone was seated around a round table:
 1. the session's objectives and programme were stated
 2. a discussion of the relevant theory took place
 3. an explanation was given of the fish evaluation procedure
- B. When seated at individual tasting booths:
 4. the fish samples were tasted.
- C. When everyone was seated around a round table:
 5. a discussion was encouraged about both the fish that had been sampled and the evaluation procedure that had been used. Refinements to the procedures were continually being implemented
 6. an instant "reward" was passed out e.g. sweets, nuts. This was also important as a means of clearing the palate
 7. the scheduling of the next session was done. While this was occurring, topics of general interest relating mainly to the fishing industry or other research projects were discussed
 8. a take-home "reward" was given as participants were departing e.g. shellfish, fruit, processed horticultural products.

In depth experience was given during training with one fish species, namely snapper. This was to develop confidence within the panelists in the hope that a positive attitude towards the sensory evaluation of fish generally would be forthcoming. However, to foster adaptability, a brief introduction to a variety of fish was also seen as being important e.g. orange roughy, hoki.

As the programme progressed, information sheets were handed out. An important aim of each session was the building of panelist's motivation and confidence. To aid this, participants were assured that all products presented for evaluation were microbiologically safe to eat. However it was also emphasised that no-one should feel compelled to eat a sample if they considered it inedible.

The effectiveness of the orientation programme was assessed by a quick "test" carried out at sessions 1 and 15. It involved presenting unannounced each individual with a whole fillet of steamed snapper and an open-ended form. This requested a description of the flavour and texture of the anterior dorsal, gut and tail regions of the fillet.

2. RESULTS AND DISCUSSION

Orientation Programme

Table I shows the terminology used by 18 panelists before and after the programme, to describe the tail region of a fillet of snapper.

With respect to flavour, the number of different terms used by the panel increased by 83%. The number of times any one descriptor was used, increased from 14 to 26%. As far as texture was concerned, there was also an increase in the range of terms being used and the number of times they were used. Similar results were obtained for the gut and anterior dorsal samples. Considering the second presentation was given without warning, the result was encouraging. Not only did the orientation programme result in an increased awareness of vocabulary and how it can be used to describe fish, but it also appeared to have a marked effect on increasing participants' confidence. They now more readily recorded their impression of the

sample. Further evidence of this latter change came from the authors' observation of participants' improved contribution to round table discussions.

TABLE I:
Descriptors given to tail region of snapper fillet
before and after orientation programme*

Flavour descriptors				Texture descriptors			
Before		After		Before		After	
sweet	7	sweet	11	moist	5	moist	7
bland	3	meaty	3	dry	4	tender	5
fishy	1	fishy	2	flakey	3	meaty	4
floury	1	chicken	2	coarse	3	chewy	4
nutty	1	salty	2	firm	2	dry	4
bitter	1	shellfish	1	dense	2	soft	4
		lamby	1	chewy	2	tough	3
		tinned peas	1	juicy	2	firm	3
		bland	1	tough	1	flakey	2
		cardboard	1	wet	1	stringy	1
		oily	1	crumbly	1	sticky	1
				melt in		creamy	1
				mouth	1	wet cardboard	
						like	1
						tendency to	
						disintegrate	1
						easy to	
						swallow	1

* Numbers apply to number of times a descriptor was recorded.

Panelist Motivation

Panelists' motivation was seen as the key to the programme's success. With this in mind, several procedures were adopted:

1. Both formal and informal contact amongst panelists, and between panelists and the authors was encouraged. Formally this was done by mixing the orientation session groups. Informally it was achieved by having morning tea after some of the sessions, by visiting for example a fish factory followed by lunch together or by having dinner together.
2. A "team" approach was fostered. Initially this was achieved by giving the group a name, SETOF. Then as the orientation programme progressed, individual strengths and weaknesses were discussed e.g. sensitivity to sulphur odours or sour tastes. Knowing these, the importance of team members being able to complement each other was emphasized.
3. The panel was kept informed about current fish research projects and the potential role of SETOF. Care was taken however not to introduce bias. Where appropriate, contact was facilitated with scientists involved in particular studies.
4. The uniqueness of the group was frequently referred to and publicity releases were made about it.
5. As part of the take-home "rewards", items that had originated from other research projects were presented. This promoted an awareness of the scope of work being done at the research centre e.g. new smoked fish products, new horticultural crops.
6. On completion of the orientation programme, a certificate endorsed by the Division's Director was presented to each team member.

7. Because of the scientific requirement for relatively frequent assessments to be made and in order to avoid "family" or other pressures on panelists regarding the cost of participating, it was decided after 2 months to offer volunteers a token payment towards the cost of travel. This was not considered by the authors to be a major factor in maintaining motivation and interest, and indeed the payment was not accepted by some members.

Use of the Panel

During the 2 years that have elapsed since the completion of the orientation programme, SETOF has continued as a highly motivated panel, capable of providing valuable information about the sensory qualities of a variety of fish and fish products.

Some examples of the type of research projects the SETOF panel have been involved with are;

1. Describing the sensory attributes of various species of fish. Table II shows an example of the type of information generated in the flavour profiles of orange roughy and hoki. Such flavour descriptions have been used to assist in the marketing of lesser known species by establishing similarities with well known species.

TABLE II:
Flavour of orange roughy and hoki fillets*

Orange roughy	Hoki
sweet	sweet
milky	milky
salty	salty
creamy	sour
lamby	lamby
nutty	bland
buttery	metallic
chicken-like	neutral
bland	chicken-like
sour	creamy
meaty	carrots
shellfish	bitter
neutral	buttery
bitter	flounder
caramel-like	fishy
	cardboard
	greasy
	soapy
	stale
	oily

- * Each descriptor was used by at least 17.5% of tasters over 6 samples.

Descriptors are in the order of frequency of use.

2. Evaluating newly developed seafood products. A range of products have been evaluated using informal round table discussions and take-home questionnaires e.g. preliminary assessments about smoked mackerel, squid products and mussel patties have been obtained.
3. Monitoring changes in the sensory properties of fish during storage and determining the shelf life. As an example the storage of orange roughy in ice was recently studied /2/. The shelf life was defined as the period of storage prior to the development of flavour, odour or texture attributes which are disliked by the panelists. In this case the description of the odour of cooked orange roughy after storage in ice was similar to that of a frozen reference

throughout the trial. Also the mean texture ratings were not significantly different from those of the reference sample throughout the storage period. The flavour, however, did change during ice storage. Table III shows the terms used by the panelists and the proportion of panelists using that term for each sampling day.

TABLE III:
Flavour of cooked orange roughy stored in ice

DAYS OF STORAGE	4		6		9		11		13	
	R**	W	R	W	R	W	R	W	R	W
Sweet	1.00*	0.67	0.76	0.76	0.79	0.50	0.68	0.58	0.88	0.18
Salty	0.27	0.20	0.41	0.35	0.36	0.29	0.32	0.32	0.24	0.18
Sour			0.18			0.29		0.26		0.24
Bitter		0.20		0.18		0.29	0.21			0.41
Lamby/Meaty	0.27	0.20	0.29	0.29	0.43			0.21	0.41	
Chicken					0.36		0.21	0.21	0.24	
Shellfish			0.18		0.21					
Milky/Creamy	0.67	0.67	0.65	0.53	0.64	0.36	0.42	0.47	0.53	0.41
Buttery	0.20		0.24	0.18	0.29		0.26		0.24	
Caramel	0.20									
Nutty	0.27		0.29	0.24			0.32		0.24	
Neutral/Bland	0.27	0.47		0.41		0.36	0.21	0.32		0.24
Mushroom						0.21				0.24
Cardboard										0.18
Potato										
Musty										0.24
Flounder								0.21		
Stale										0.29
Metallic						0.36				0.18

* Figures are the proportion of tasters using a flavour term.
Proportions were recorded if greater than 0.175.

** R - Reference; frozen fillets
W - Whole, stored in ice

The flavour of the frozen reference sample after cooking was consistently described by the 12-20 members of the SETOF panel as milky/creamy, sweet, lamby/meaty, salty, nutty and buttery. Because it did not change markedly over the storage period, it provides evidence that the panelists, who were not aware of the inclusion of a reference sample, were consistent in their assessment of cooked flavour.

Like the reference sample, the ice stored orange roughy after cooking were frequently described as sweet, salty, milky/creamy and lamby/meaty. However bitter, sour and neutral/bland descriptions were also included as time elapsed. By day 13, the iced fish had developed mushroom, cardboard, musty, stale and metallic flavours. There was also a decrease in the proportion of tasters using the term sweet from 58% on day 11 to 18% on day 13. These changes coincided with the acceptability rating for flavour showing a significant drop on day 13 for the iced fish. This suggests that the mushroom, cardboard, musty, stale and metallic flavours and the loss of sweetness described by the panel were disliked. As defined previously the shelf life of orange roughy in ice can be considered as the period that has expired prior to the development of aspects which are disliked by the panelists. In this trial the shelf life of whole orange roughy in ice was therefore between 11 and 13 days. The shelf life was more influenced by flavour change than by changes in odour or texture.

3. CONCLUSIONS

A panel was selected and trained in order to obtain data on the eating quality of fish products. The panel lacked the bias commonly found when research colleagues are

used and yet they were consistent and reliable in their evaluations. The panel members were highly motivated by a training programme which gave them new social opportunities and confidence as assessors.

A panel of this type can provide information about the sensory attributes of fish. In particular the changes that occur during storage of fish can be examined and the shelf life determined. Results of the descriptive sensory evaluation can then be used to decide which chemical or microbiological tests are appropriate for the determination of shelf life in that product.

REFERENCES

1. HOGG, M.G. and SCOTT, D.N. 1984. Selection and training of a New Zealand taste panel on fish. Fish Processing Bulletin Number 1, Division of Horticulture and Processing, DSIR, Auckland.
2. SCOTT, D.N., FLETCHER, G.C., HOGG, M.G., RYDER, J.M., SUMMERS, G. and SEELYE, R.J. 1984. Storage Characteristics of Orange Roughy (Hoplostethus atlanticus) Held in Ice. Fish Processing Bulletin No. 3, DSIR. Mt. Albert Research Centre, Auckland, New Zealand.

FORMATION ET UTILISATION D'UN JURY DE DEGUSTATION POUR LA DETERMINATION DE LA CONSERVATION DU POISSON EN MAGASIN DE DETAIL.

RESUME : Cet article décrit la formation d'un jury de dégustation pour l'évaluation sensorielle du poisson. Les méthodes de sélection des membres du jury en dehors du centre de recherche sont exposées. Les membres du jury sont formés dans le cadre d'un programme d'orientation destiné à accroître leur confiance et leur motivation. Les moyens de maintenir la motivation sont examinés et l'utilisation du jury est décrite.

Le jury mis en place n'a pas souffert des difficultés ordinairement liées aux groupes de ce genre : assiduité médiocre, préjugé causé par une connaissance préalable, manque de motivation et de souplesse.

On utilise Hoplostethus atlanticus (famille des trachichthyidés) conservé dans de la glace pour illustrer les avantages de ce jury en ce qui concerne la détermination de l'appétit à la conservation.

DIFFERENTIAL SCANNING CALORIMETRY
OF FROZEN COD: pH EFFECTS

R. HASTINGS, G. RODGER*
Unilever Research Laboratory, Torry, Aberdeen (United Kingdom)

1. INTRODUCTION

DSC is a useful means of studying the thermal properties of intact proteins without the need for solubilisation of the sample. The DSC thermogram of whole cod muscle has been described in terms of the constituent proteins; the peaks and shoulders of the thermogram can be assigned to one or more of myosin, actin, sarcoplasmic proteins or collagen (1). The technique has been used to study the effects of processes such as frozen storage, salting, marinating and dehydration on the constituent proteins of cod muscle. The myosin component was shown to be the least stable to increasing temperature and easily irreversibly changed in structure during handling and processing.

The pH of fish muscle has a well-known effect on the texture and storage life of fish products (2). Thermograms of cod muscle over a range of pH 5.0 to pH 8.0 were recorded in an attempt to interpret the effects of pH in terms of changes in individual muscle proteins.

2. METHODS

pH Adjustments

Fresh skinned North Sea cod fillets were minced and divided into seven portions. Samples were mixed with an equal weight of distilled water and were adjusted with 1.0 M HCl or 1.0 M NaOH to the following pH values; 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0. Constant ionic strength was maintained with 1.0 M NaCl. Samples were allowed to equilibrate for 20 hours at 4 °C before checking and re-adjusting the pH values. Excess fluid was allowed to drain from the minced fish.

pH-adjusted samples were blast frozen immediately and stored at -40 °C until analysed. Samples of pH 5.5, 6.5 and 7.5 were also stored at -10 °C and were examined after, 2, 4, 8 and 12 weeks.

Differential Scanning Calorimetry

DSC was performed on a Perkin-Elmer DSC II. Samples weighing 10-15 mg were sealed in Perkin-Elmer aluminium sample pans. Reference pans contained an equal weight of water. The samples were heated at 10 K/minute over the range 274 K-370 K at an instrument sensitivity of 0.1 or 0.2 mcal/second. The apparent heat of transition, ΔH_{app} , was determined from the peak area using a conversion factor obtained from an Indium standard and expressed in mcal/mg dry weight. The peaks in the thermogram were recorded to mark transition temperatures (T_m) for individual muscle proteins.

3. THE EFFECT OF pH ON COD MUSCLE

Thermograms of samples in the pH range 5.0 to 8.0 displayed two major transitions (e.g. 317.7 ± 0.5 K and 349.2 ± 0.4 K at pH 6.5) which have been assigned previously to myosin and actin respectively. The highest transition temperature for actin occurred at pH 5.5 near the isoelectric point (Fig. 1). As the pH was raised above or below this, the transition temperature fell e.g. from 350 K at pH 5.5 to 343 K at pH 8.0. The protein was progressively more susceptible to thermal denaturation as it became increasingly net charged. Increasing the pH from 5.0 to 8.0 led to a decrease in the observed T_{max} of the transition assigned to myosin. Similar effects of pH on the thermal denaturation of myosin and actin were found in beef by Stabursvik and Martens (3).

* Present address: Bioproducts Group, ICI Agricultural Division,
Billingham, Cleveland (England)

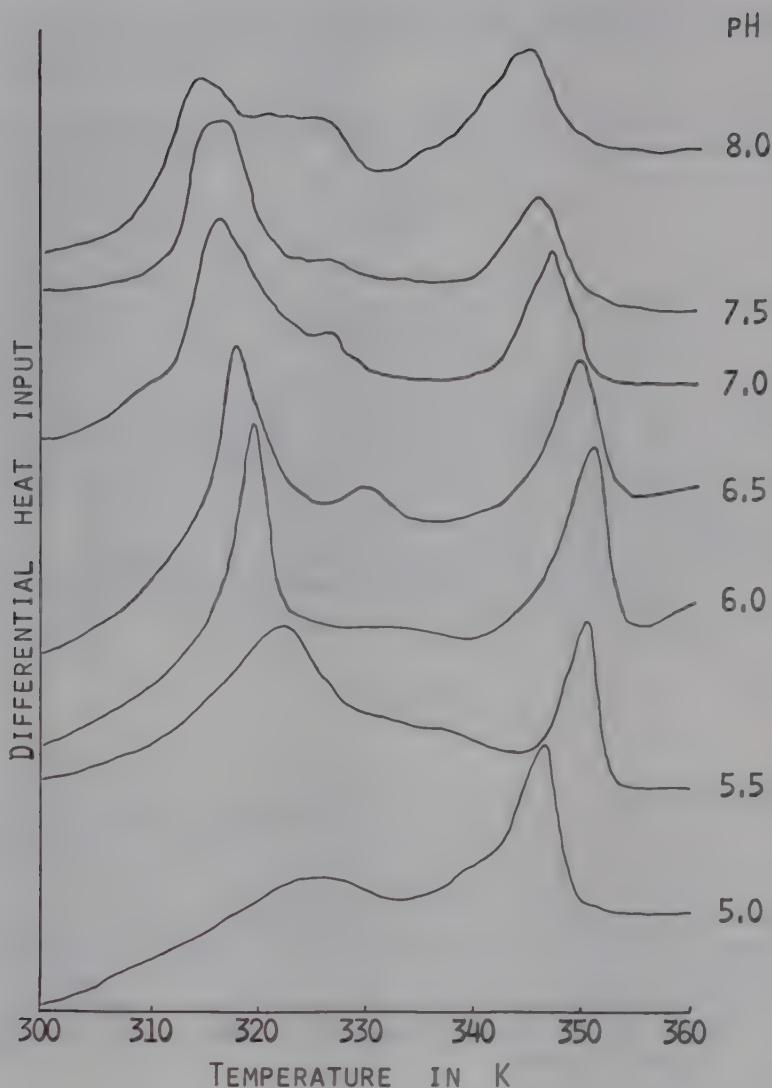


Fig. 1 - DSC thermograms of cod muscle with adjusted pH values

4. FREEZE DENATURATION OF COD MUSCLE

Cod muscle at pH 5.5, 6.5 and 7.5 were examined during frozen storage at -10°C . In each case, the major myosin peak at $\sim 318\text{ K}$ decreased in size on the profile within about 4 weeks. Fig. 2 gives an example of thermograms at various storage intervals for one pH value. The loss of the major myosin peak and broadening of the profile is probably correlated with aggregation of the muscle proteins and loss of salt solubility (4).

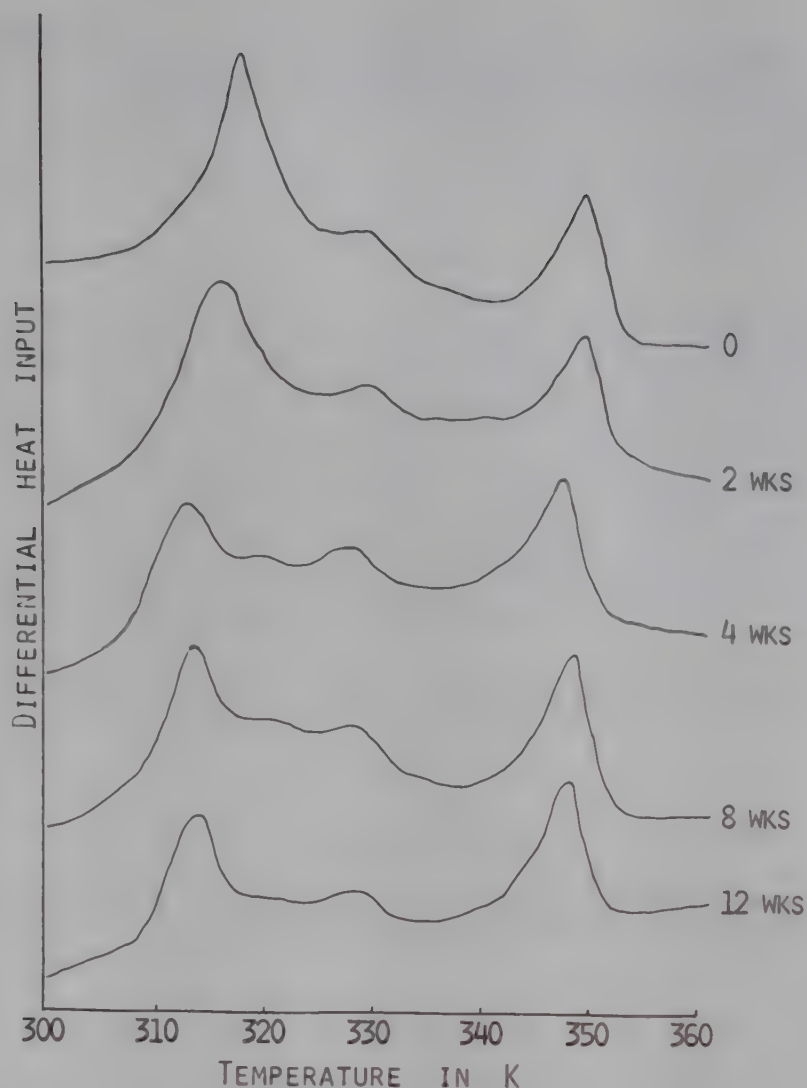


Fig. 2 - DSC thermograms of cod muscle adjusted to pH 6.5 during 12 weeks frozen storage at -10°C .

Higher enthalpy values were recorded at pH 6.5 and pH 7.5 than at pH 5.5 even after storage, (Table 1). Cod muscle proteins are normally in an environment of pH 6.3 to 7.2 and at these pH values high enthalpy values were recorded.

TABLE 1

Average Apparent Heat of Transition (ΔH_{app})
of pH-adjusted Cod Muscle during Frozen Storage at -10°C

Storage Time	pH 5.5	pH 6.5	pH 7.5
Zero	2.65 ± 0.30	3.33 ± 0.11	3.00 ± 0.28
2 weeks	2.79 ± 0.31	3.50 ± 0.39	3.96 ± 0.10
4 weeks	1.95 ± 0.58	3.33 ± 1.16	3.13 ± 0.27
8 weeks	1.80 ± 0.17	3.17 ± 0.69	3.34 ± 0.73
12 weeks	2.55 ± 0.38	2.70 ± 0.98	2.82 ± 0.26

There was a trend for mean values of apparent heat of transition to fall slightly in each case with increasing time of frozen storage (Table 1). After the major myosin peak reduced in size on the thermogram, at about 4 weeks, there was no significant change.

5. CONCLUSIONS

DSC has been used to examine the effects of pH adjustment on cod muscle and subsequent changes during frozen storage. The thermal denaturation of the constituent proteins of cod muscle showed a dependence on pH, with the highest transition temperatures for myosin and actin occurring at pH 5.0 and 5.5 respectively, near the isoelectric point of the myofibrillar proteins. The apparent enthalpy of denaturation of cod muscle did not show a clear relationship with pH, though after frozen storage muscle proteins at higher pH values had higher enthalpy values. This finding may be related to the known resistance to frozen storage denaturation of high pH value fish (2). The natural pH of cod muscle may fall between pH 6.3 and 7.2. A relationship of enthalpy of denaturation with pH has been demonstrated in rabbit myosin (5). However, in this study variation in samples and difficulties in baseline interpretation prevented accurate determination of enthalpy relationships.

This technique can provide a valuable insight into the molecular changes in fish muscle proteins during freeze denaturation and the potential storage life of the raw material.

ACKNOWLEDGEMENTS

The authors would like to thank Mr. R.B. Weddle and Mr. P. Wilding, Unilever Research Laboratory for advice and discussion of the work.

REFERENCES

1. R.J. HASTINGS, G.W. RODGER, G.R. PARK, A.D. MATTHEWS, E.M. ANDERSON. "Differential Scanning Calorimetry of Fish Muscle: The Effect of Processing and Species Variation". J. Food Sci., Vol. 50, No. 2, pp. 503-506, 510 (1985).
2. R.M. LOVE. "The Chemical Biology of Fishes". Vol. 2: Advances 1968-1977. Academic Press, p. 360 (1980).
3. E. STABURSVIK and H. MARTENS. "Thermal Denaturation of Proteins in Post Rigor Muscle Tissue as Studied by Differential Scanning Calorimetry". J. Sci. Food Agric. 1980, 31, 1034-1042.
4. G.W. RODGER, R.B. WEDDLE and P. CRAIG. "Effect of Time, Temperature, Raw Material Type, Processing and Use of Cryoprotective Agents on Mince Quality". Adv. in Fish Science and Technology, 23-27 July, 1979. Ed. J.J. Connell. Pub. Fishing News Books Ltd.
5. D.J. WRIGHT and P. WILDING. "Differential Scanning Calorimetric Study of Muscle and its Proteins: Myosin and its Subfragments". J. Sci. Food Agric. 1984, 35, 357-372.

LA CALORIMETRIE A BALAYAGE DIFFERENTIEL DU CABILLAUD CONGELE : EFFETS DU PH.

RESUME - La calorimétrie à balayage différentiel est un moyen intéressant pour l'étude des propriétés thermiques des protéines du muscle et leurs transformations au cours de l'entreposage à l'état congelé. Des thermogrammes de muscle de cabillaud ont été enregistrés pour des pH de 5.0 à 8.0 à concentration ionique constante. La dénaturation thermique des protéines de constitution du muscle de cabillaud a montré qu'elle dépendait du pH, les températures maximales de transition apparaissant au voisinage du point iso-électrique des protéines myofibrillaires. On a étudié des échantillons de muscle de cabillaud à des pH de 5,5, 6,5 et 7,5 au cours d'un entreposage de 12 semaines à -10°C. Dans chaque cas, une valeur importante de pointe de la myosine

diminuait en moins de 4 semaines environ et les valeurs de l'enthalpie avaient tendance à s'abaisser légèrement avec la durée de l'entreposage. Dans cette étude, les poissons à pH plus élevé avaient des enthalpies générales plus élevées après entreposage, ce qui peut être dû à leur résistance connue à la dénaturation pendant leur entreposage à l'état congelé. Cette technique peut donner une connaissance précieuse sur le degré de dénaturation par congélation des protéines musculaires du poisson et la durée de conservation possible de la matière première.

PREVENTION OF BLACK SPOT IN RETAIL-PACKED FROZEN NORWAY LOBSTERS

B. Jessen
N.C. Jensen

Technological Laboratory, Danish Ministry of Fisheries
Technical University
Lyngby (Denmark)

The main part of the Danish catch of Norway lobsters (Nephrops norvegicus) is exported, especially to Southern Europe, Italy being the most important export market. Usually sulfite is added to Nephrops to prevent development of black spot. This blackening is due to enzymic oxidation of the monophenol tyrosine to quinones followed by polymerization to melanins, which results in Nephrops of inferior quality.

From time to time and for different reasons, additives are banned or restricted, also as regards sulfite in frozen Nephrops. With the purpose of finding an alternative to sulfite treatment, investigations were implemented to evaluate the effect of various treatments and packing methods. Based on industry requirements, Nephrops were to have a storage life of 1 year at -30°C followed by 4 days at 5°C .

2. MATERIALS AND METHODS

Codes

The following treatments and packing methods were considered:

- | | | |
|-----------------------|---|---|
| Control | - | untreated |
| Blanching | - | B85-10, B85-20, B100-5, B100-10 - heat treatment for 10 and 20 seconds at 85°C and for 5 and 10 sec. at 100°C , respectively. |
| Sulfite | - | 0.5%S and 1.0%S - the figures indicate the concentration (w%) of sodium bisulfite (NaHSO_3) in water. |
| Citric acid | - | 1% C and 5% C - the figures indicate the concentration (w%) of citric acid in water. |
| Ascorbic acid | - | 1% A and 3% A - the figures indicate the concentration (w%) of ascorbic acid in water. |
| Controlled atmosphere | - | N_2 - packed in nitrogen (N_2). Packing materials were PVC/PEP (O_2 -permeability = $2.4 \text{ cm}^3/\text{m}^2/24 \text{ hr}/1 \text{ atm}_2$) and PETP/PE/PVDC (O_2 -permeability = $10-20 \text{ cm}^3/\text{m}^2/24 \text{ hr}/1 \text{ atm.}$). |

The samples treated with sulfite, citric acid and ascorbic acid were prepared by dipping Nephrops in brine for 10 minutes. Density was 1-2 Norway lobsters per litre of brine.

Raw materials

The Nephrops were caught during a 12 hour voyage in the Kattegat in June and iced at landing. Before sample preparation the Nephrops were graded and damaged Nephrops and Nephrops with black spot or incipient black spot were rejected.

After treatment the Nephrops were double-glazed and stored at -30°C .

Methods

Tests were carried out after 4 days, 2 weeks, 1 month, 3 months, 6 months and 12 months storage.

Visual evaluation: The precursors of the formation of black pigments are mainly located on the cephalothorax and near the periphery of the tail. The blackening begins on both sides of the thorax and later on

spreads to the head, claws - especially in the joints - legs and abdomen.

For visual evaluation, 20 Norway lobsters were thawed and then placed at 5°C. They were judged by the naked eye and placed in categories according to the extent of blackening:

- Stage I: Normal, no black spot at all
- Stage II: Development of black spot on the cephalothorax
- Stage III: Cephalothorax, tail etc. black.

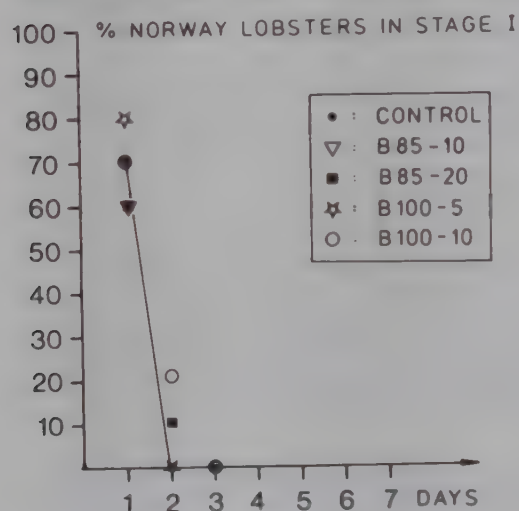
Sensory assessment: The organoleptic quality was estimated under code by a panel of 6-8 members. Numerical scores for odour, flavour and texture were given on a scale where 10 represents the ideal product and 0 the lowest quality while 4 indicates the limit of acceptability.

Chemical assessment: Nephrops were examined for total volatile nitrogen (TVN) /1/, pH, dry matter, salt and sulfite /2/, /3/.

3. RESULTS

Visual evaluation

Thermal inhibition: The time/temperature combination was limited by the fact that the lobsters were to retain a fresh appearance. In accordance with this definition, the combinations chosen were found to be the maximum possible. The heat-treated codes were followed for only one month. Very soon it became evident that discoloration was accelerated in the heat-treated codes as compared with the control (fig. 1).



Inactivation of the enzyme system responsible for discoloration apparently requires a heat treatment that gives the Norway lobster a cooked appearance due to protein coagulation. This phenomenon is easily detected on the abdomen membrane that turns opaque upon cooking.

Fig. 1 - Percentage of Norway lobster (stored 1 month at -30°C) in stage I after storage at 5°C.

Chemical inhibitors

Citric acid: Neither treatment with 1% C nor with 5% C delayed the development of black spot. Besides, citric acid, especially the high concentration, caused precipitation of white crystals on the surface of the lobsters. The evaluation of these codes was stopped after one month frozen storage.

Ascorbic acid: A preliminary investigation showed a small delay in the occurrence of discoloration. However, the treatment with 3% ascorbic acid was not followed for more than one month as it caused a yellow appearance.

As can be seen from figs 2 and 3 1% A was slightly better than the control without resulting in a significant improvement. The effect of ascorbic acid is debatable. Some authors claim a reducing effect on quinones, which inhibits quinone oxidation /4/, while others find that reducing compounds like ascorbic acid increase the reaction rate of monophenol to diphenol /5/.

Sulfite: Sulfite concentrations higher than the ones used left the Nephrops with a pronounced sulfite flavour and odour after cooking. The occurrence of discoloration was considerably delayed with a sulfite treatment of Nephrops. Within the storage period required by the industry, a sulfite treatment of 0.5% sulfite was comparable to one of 1.0% sulfite, see figs 2 and 3. After 2 days at 5°C all Nephrops generally had an acceptable appearance and then discoloration took place more frequently although the appearance still was regarded as acceptable after 4 days. After 12 months at -30°C there was a slight tendency towards an earlier start of discoloration while no differences could be observed up to 10 months of storage.

The mechanism behind the effect of sulfite is not fully explained but is believed to be due to a reaction between sulfite and quinones whereby polymerization to melanins is inhibited [6/].

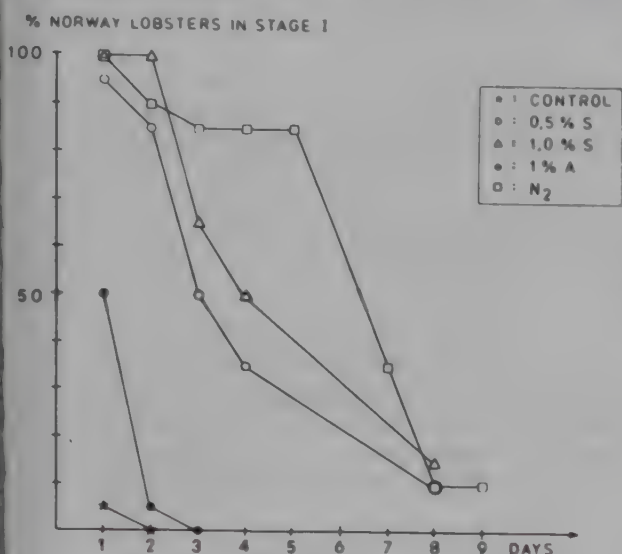


Fig. 2 - Percentage of Norway lobsters (stored 2 weeks at -30°C) in stage I after storage at 5°C.

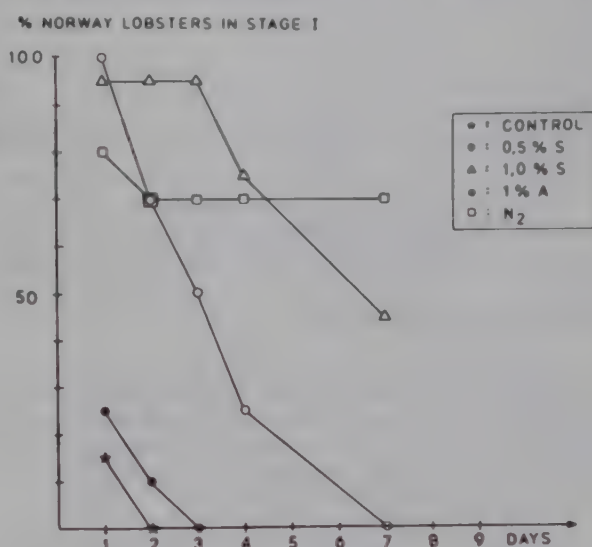


Fig. 3 - Percentage of Norway lobsters (stored 10 months at -30°C) in stage I after storage at 5°C.

Controlled atmosphere: From preliminary experiments, vacuum-packing was excluded as a packing method for Norway lobsters due to perforation of the plastic material by claws and the rostrum.

Instead, oxygen-free surroundings were achieved with nitrogen packing, the visual evaluation of which can be seen in figs 2 and 3. Generally, the N₂-code remained longer without development of black spot than the other codes. This was particularly marked after 3-4 days storage as there was no onset of discoloration in 55-85% of the packages. Head space analyses revealed that black spot on Nephrops was caused by leakages, very often in the welding seam. Another problem turned up at the end of the storage period. After 12 months at -30°C about half of all packages were not tight, indicating an oxygen permeable film.

Sensory assessment

No difference could be observed between the codes during the storage period and all Nephrops were judged acceptable. After one year of storage they were acceptable at a level of 6 to 7 on the 10-point scale, fig. 4. The shell of freshly thawed Nephrops was pink without any sign of black spot. Odour and flavour of cooked Nephrops were judged neutral to crustacean-like and no off-flavours from the sulfite- and ascorbic-acid treated codes could be detected. Generally, the texture was firm but from time to time soft-textured Nephrops appeared. This condition is attributable to water absorption during the molting phase.

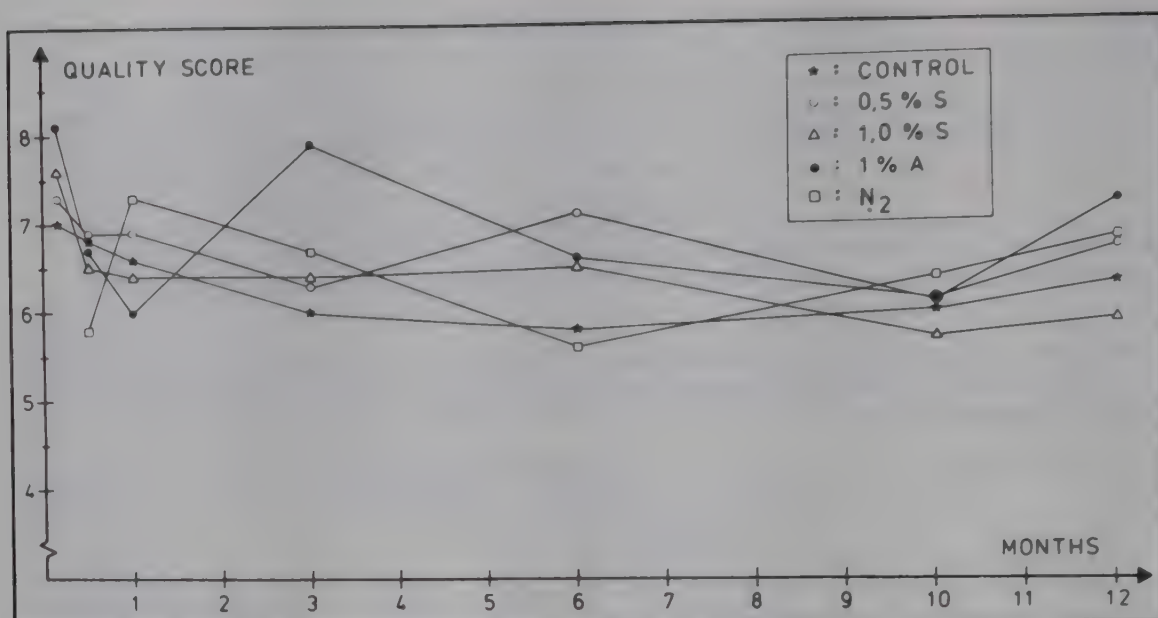


Fig. 4 - Organoleptic quality of cooked Norway lobster tails after storage of up to 1 year at -30°C .

Chemical assessment

The results from the chemical analyses of raw material are given in table 1. Spot tests throughout the storage period revealed no changes in pH, dry matter and salt. TVN fluctuated between 13 and 25 mg/100 g. Taking into consideration the high level of non-protein nitrogen compounds in Nephrops flesh, the TVN-values indicated Nephrops of good quality /7/, /8/.

Table 1 Chemical data of fresh untreated Norway lobsters

pH in shell	8.4
pH in flesh	6.6
dry matter	19.4%
salt	0.7%
TVN	18 mg N/100 g
sulfite in shell	0 mg SO_2/kg
sulfite in flesh	0 mg SO_2/kg

Sulfite: Detection level of the sulfite analysis is 30 mg SO_2/kg , and as seen from table 2, the sulfite concentration in lobster mince never exceeded this level.

Table 2 - SO_2 -level (calculated as the average of 10 codes) in sulfite-treated Norway lobsters

Code	SO_2 -content mg SO_2/kg in			
	shell	min.	- max.	flesh
1.0 S	380	270	- 810	approx. 30
0.5 S	220	20	- 370	approx. 30

Due to a wide variation within the codes and also to a marked deviation (10%) in the analytical results, no significant change in the sulfite concentration was observed during storage, although vaporization was thought to cause a drop in the SO_2 -level. The sulfite concentration in the flesh was approximately one tenth of the shell concentration. According to the Danish regulations, 30 mg SO_2/kg is permitted in the edible part of crustaceans /9/. The sulfite-treatments, 0.5%S and 1.0%S were within this limit.

4. DISCUSSION

The black spot noticed on Norway lobsters is assumed to be an oxygen-dependent enzyme-catalyzed reaction. Neither flavour nor texture is influenced by the reaction and as such the discoloration is an aesthetic problem and not a question of deteriorated Nephrops. Only the initial stages in the development of black spot are enzyme-catalyzed, whereas the polymerization of quinones to melanin is non-enzymic and proceeds spontaneously /10/, /11/, /12/.

The melanin formation is closely related to sclerotization as the precursors are the same /12/. Prior to molting, the precursors accumulate and the tendency to blackening is high, whereas melanosis seldom is seen in the sclerotization period. Injured Nephrops also show a higher tendency to blackening, partly due to access of free oxygen and partly due to accumulation of precursors (sclerotization) /13/.

Treatment to prevent black spot should be started immediately after death in order to obtain a proper inhibition. Based on the present results, the following methods to prevent discoloration can be mentioned:

Glazing Double-glazing is sufficient to protect against blackening during freezer storage but does not fulfil industry requirements as discoloration appears within 24 hours after thawing. It is the cheapest treatment of all but implies the disadvantage of a radical change in consumption pattern, marketing etc. as the Nephrops must be cooked direct from the frozen stage.

Controlled atmosphere: Packing in nitrogen-atmosphere is an alternative to sulfite treatment and even results in a slight improvement of the storage life. The disadvantages are the expense and an inevitable change in marketing, as nitrogen-packed Nephrops are as perishable as untreated as soon as the package is opened. Besides, technological problems like development of a plastic material with a lower oxygen permeability, leaking glazing coat inside the package and avoidance of antennae in the welding seam have to be solved.

Sulfite: Sulfite treatment is easy, cheap and effective, fulfilling the industrial requirements. Treatment of 10 minutes in a 0.5% sulfite solution sufficiently prevents development of black spot and does not cause off-flavour in Norway lobsters.

5. CONCLUSION

Ways to prevent development of black spot in frozen Norway lobsters were examined by treatments with chemical inhibitors and by physical methods. Industry requires a storage life of 1 year at -30°C followed by 4 days at 5°C . Based upon this it is concluded that the only alternative to the traditional sulfite treatment is packing in nitrogen atmosphere. Some technological problems, however, still have to be solved and in addition, the method implies a change in marketing and increased production costs. In comparison, it has been shown that dipping for 10 minutes in a 0.5% sulfite solution is effective within the requirements mentioned.

REFERENCES

1. E.J. CONWAY & A. BYRNE: An Absorption Apparatus for the Micro-determination of Certain Volatile Substances. I. The Microdetermination of Ammonia. J. Biochem. (1933), 27, pp. 419-429.
2. J. SHIPTON: Estimation of Sulphur Dioxide in Dried Foods. C.S.I.R.O. Food Preservation Quarterly (1958), 14(2) p. 54.
3. NORDISK METODIK KOMITE FOR LEVNEDSMIDLER: Bestemmelse af svovlsyring (1954), No. 18.

4. N.A.M. ESKIN, H.M. HENDERSON & R.J. TOWNSEND: Biochemistry of Foods. Academic Press, New York/London (1971).
5. S.P. COLOWICK & N.O. KAPLAN: Methods in Enzymology, Vol. II. Academic Press Inc. Publishers, New York (1955).
6. R.J. EMBS & P. MARKAKIS: The Mechanism of Sulphite Inhibition of Browning Caused by Polyphenol Oxidase. J. Food Sci. (1965), 30, pp. 753-758.
7. P. WALKER, D. CANN & J.M. SHEWAN: The bacteriology of "Scampi" /Nephrops norvegicus). I: Preliminary bacteriological, chemical and sensory studies. J. Fd. Technol. (1970), 5, pp. 375-385.
8. G.D. STROUD, J.C. EARLY & G.L. SMITH: Chemical and sensory changes in iced Nephrops norvegicus as indices of spoilage. J. Fd. Technol. (1982), 17, pp. 541-551.
9. STATENS LEVNEDSMIDDELINSTITUT: Fortegnelse over godkendte tilsætningsstoffer til levnedsmidler. Miljøministeriet (1983), publ. No. 80.
10. H.S. MASON: Structures and Functions of the Phenoles Complex. Nature (London) (1956), 177, pp. 79-81.
11. H.S. MASON, W.L. FOWLKS & E. PETERSON: Oxygen Transfer and Electron Transport by the Phenolase Complex. J. Amer. Chem. Soc. (1955), 77, pp. 2914-2915.
12. B.F. COBB III: Biochemistry and Physiology of Shrimp - Effect on Use as Food. In "Proceedings of the Conference on the Handling Processing and Marketing of Tropical Fish". Tropical Products Institute, London (1977).
13. N.M. SUMMERS: Cuticle Sclerotization and Blood Phenol Oxidase in the Fiddler Crab, Uca pugnax. Comp. Biochem. Physiol., (1967), 23, pp. 129-138.

PREVENTION DES TACHES NOIRES DANS LES LANGOUSTINES CONGELEES, EN EMBALLAGES DE DETAIL

RESUME: Des langoustines (Nephrops norvegicus) congelées, en emballage de détail, ont été traitées de façon traditionnelle avec du sulfite pour inhiber le développement de taches noires. En raison, en partie, des restrictions à l'importation et, en partie, des effets toxiques, on a examiné d'autres méthodes de conservation. On a tenu compte des paramètres suivants pour éviter le noircissement : traitement thermique (blanchiments), acide ascorbique, acide citrique, bisulfite de sodium (NaHSO_3) et emballage sous gaz (N_2).

D'après les besoins de l'industrie, les langoustines devaient avoir une durée de conservation correspondant à un an à -30°C , suivie de 4 jours à 5°C , sans apparition d'une couleur anormale ni détérioration. Les seuls traitements répondant à cette exigence étaient ceux au bisulfite de sodium et l'emballage sous azote, tandis que les autres traitements ont échoué. Non seulement les taches noires n'ont pas été évitées, mais les langoustines ont été rejetées pour d'autres raisons, telles que la coagulation des protéines (blanchiment), le jaunissement (acide ascorbique) et la formation de cristaux blancs (acide citrique). Le traitement au sulfite est la méthode la plus commode et en même temps le meilleur marché, une immersion de 10 min dans 0,5 % en masse de bisulfite de sodium étant suffisante. A l'inverse, l'emballage sous azote est coûteux, et, de plus, exige une commercialisation active. De plus, des problèmes technologiques tels que la formation de liquide par suite du givrage et le fait d'avoir à éviter d'introduire les antennes dans les soudures restent à résoudre.

PREVENTION OF BROWNING DISCOLORATION AND DRIP LOSS OF FROZEN SHUCKED OYSTER (Crassostrea gigas)

SHANN-TZONG JIANG, CHING-YU TSAO AND TUNG-CHING LEE .
Department of Marine Food Science, National Taiwan College of Marine
Science & Technology, Keelung, Taiwan 200

INTRODUCTION

Most oysters harvested in oriental countries such as Japan, Korea, China etc are cultured oysters. They are a favourite marine food for the oriental people, especially for Chinese, due to the special fishy flavour and tender texture /1,2,3/. They are an important exported marine product for Japan /4/. In addition, the high content of polyunsaturated fatty acids in this species make it a suitable food for atherosclerosis patients /5/.

In France, large scale farming of oyster (Crassostrea japonica) in the open sea has been developed and the production is expected to rise from 3000 tons per year to 15,000 tons per year by 1985 /6/. In the United States, it is estimated that there are about 1,400,000 acres of bottom in territorial water of this country designated as oyster producing areas. There are three important species there; i.e. eastern oyster (Crassostrea virginica), Pacific oyster (Crassostrea gigas) and Olympia oyster (Ostrea lurida) /7/.

In order to supply a good quality oyster to the inland markets throughout the year, an efficient preservation technique is necessary. The shucked oyster was still acceptable after 20 days and 10 days storage at -2°C and $5-7^{\circ}\text{C}$ respectively /8/. The live Pacific oyster could also keep for at least 17 days and 13 days at 0°C and $2-3^{\circ}\text{C}$ respectively /9/. However, the raw shucked oyster lost their original flavour and deteriorated in taste after 3 days ice storage /10/. This storage life is still not long enough for distribution inland for consumption.

Freezing is one of the most satisfactory methods currently available for long term preservation of foods. Several reviews on the technique of freezing oysters were made by Hansen and Aagaard /11/, and Yamasaki /4,12/. Although the oyster has been frozen in a variety of ways as seen in the supermarkets in Japan and U.S., the frozen oyster still has some unsolved problems such as over 20% of drip loss during thawing /3,11/, and browning discoloration during prolonged storage /3,7/. The prevention of drip loss of frozen shucked oyster on defrosting was studied by some researchers /2,3,4,13/. However, the browning discoloration is still not resolved. This study was carried out in order to prevent the discoloration and subsequently reduce drip loss for improving the quality of frozen shucked oyster.

MATERIALS AND METHODS

All fresh shucked oyster (Crassostrea gigas) used in this study were obtained from a commercial oyster-culture farm, in Southern Taiwan. They were collected from beds and kept alive in a sea water tank, and transported to laboratory immediately. Oyster was shucked by inserting a special knife between shells to remove the meat. After shucking, washing and draining, the fresh oyster was then used for the following experiments.

A). Determination of the inhibitory effect of various antioxidants on browning discoloration:

The fresh shucked oyster was dipped in various antioxidant solutions (Table I) for 15 minutes at a solution temperature below 5°C . After draining the excess dip solution, samples were placed on a stainless steel tray, covered with polyethylene film and stored at 1°C for 10 days. At definite time intervals, samples were removed and subjected to the following assays to evaluate the inhibitory effect of these antioxidants on discoloration.

Thiobarbituric acid (TBA) test: The development of rancidity during storage was determined by TBA test. The TBA value was measured according to Yu and Sinnhuber /14/.

I.I.F.-I.I.R.-Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

The TBA value was expressed as milligrams of malonaldehyde per kilogram of sample.

TABLE I Antioxidants used in immersion treatment

treatment	antioxidant
I	control
II	3% NaCl + 1% ascorbic acid
III	3% NaCl + 1% Na-ascorbate
IV	3% NaCl + 1% erythorbic acid
V	3% NaCl + 1% Na-erythorbate
VI	3% NaCl + 0.01% ethylenediamine Tetraacetic acid
VII	3% NaCl + 0.01% butylated hydroxyanisole

Determination of the acid value (A.V.): The lipid of oyster was extracted with chloroform:methanol = 2:1. After filtering the tissue, solvents were evaporated using a vacuum evaporator. The A.V. of the lipid obtained from the oyster meat was determined according to Shinma /15/, and expressed as milligrams of potassium hydroxide, needed to neutralize the free fatty acids, per kilogram of oyster sample.

Determination of indole content: The content of indole in oyster meat was a very good indicator for evaluating the quality of oyster /16/. In this experiment, the content of indole in oyster meat was measured according to AOAC /17/.

Sensory evaluation: The sensory evaluation was performed using a 9-point hedonic scale by 6 trained panellists. The degree of discoloration from 500 grams oyster samples was also recorded by the panellists and expressed as: A: natural colour; B: slightly browning discoloration occurred; C: browning discoloration occurred; D: dark discoloration occurred.

B). Effect of some treatments before freezing and after freezing on preventing the discoloration and drip loss of oyster during frozen storage:

According to the previous study /3/, immersion treatment with 3% sodium chloride solution before freezing showed excellent effects on reducing the drip loss of frozen shucked oyster. Mixtures of 3% salt and some antioxidants (i.e. Na-erythorbate, erythorbic acid and Na-ascorbate) which effectively inhibited the oxidation discoloration were employed.

TABLE II. Composition of the solutions used in various treatments

treatment	composition
I	control
II	3% NaCl + 1% Na-erythorbate
III	3% NaCl + 1% erythorbic acid
IV	3% NaCl + 1% Na-ascorbate
V	3% NaCl + 0.5% erythorbic acid + 0.5% Na-erythorbate
VI	3% NaCl + 0.5% Na-erythorbate + 0.5% Na-ascorbate
VII	3% NaCl + 0.5% Na-ascorbate + 0.5% erythorbic acid

Immersion treatment before freezing: The immersion solutions were prepared by dissolving the antioxidants into the 3% sodium chloride solution which was prechilled to a solution temperature below 5°C (Table II). After shucking, washing and draining the oysters, samples were immersed in these solutions for 15 minutes. After draining the excess dip, samples were quickly frozen (I.Q.F.) to a temperature below -18°C using an air blast freezer (-40°C, 3 m/sec) and stored in a -20°C freezer for a year.

Ice glaze treatment after freezing: After freezing, shucked oyster samples were glazed to the extent of about 4-5% of body weight by immersing twice in the glazing solutions (Table II) for 5 seconds each. The glazed samples were stored in a -20°C freezer for a year.

Combination treatments utilizing both the immersion pretreatment and ice glaze treatment: Samples immersed in the combined mixture solution of 3% sodium chloride and antioxidants (Table II) were individually quick frozen to a temperature below -18°C using an air blast freezer. The samples were glazed immediately using the solution listed in Table II. The glaze was about 4-5% of the body weight of oyster. After these treatments, samples were stored in a -20°C freezer for a year.

Quality assessments: The changes in the quantity of drip loss on thawing and organoleptic quality was monitored to evaluate the effect of the treatments cited above on

the quality of frozen shucked oysters.

Determination of the drip loss: The quantity of free drip loss upon thawing was measured:- about 400-600 grams of frozen oyster was weighed and placed on a funnel covered with cheese cloth and fitted in a 250 ml measuring cylinder. A thermocouple thermometer was used to measure the body temperature of oyster during thawing. After the temperature of the sample reached 5°C, the free liquor was weighed, corrected for the quantity of glaze added and expressed as a percentage of the weight of the glazed oyster before thawing.

RESULTS AND DISCUSSION

Effect of the antioxidant immersion treatments on the development of rancidity of oyster during storage:

The changes in the acid value and TBA value of the treated oyster during storage at 1°C are shown in Figures 1 and 2. Samples treated with BHA showed less change in acid value during 10 days storage than those treated with the water-soluble antioxidants. From Figure 2, the TBA value of the control and treated samples increased gradually with prolonged storage. The sample treated with BHA showed the lowest value after 10 days storage at 1°C.

Effect of antioxidant immersion treatments on indole content and organoleptic quality of oyster during storage: As shown in Figure 3 and Table III, the change in indole content of oysters in each group showed similar increasing patterns during 10 days storage at 1°C. No effect of these antioxidants on preventing the development of indole was observed during storage.

According to organoleptic quality shown in Table III, samples treated with ascorbic acid (II), Na-ascorbate (III), erythorbic acid (IV) and Na-erythorbate (V) were slightly discoloured after 10 days storage. However, samples of control, treated with EDTA and BHA were darkly discoloured after 10 days storage at 1°C. All samples, except those treated with EDTA and BHA were still acceptable after 10 days storage (Table III). The sample treated with BHA, especially, produced very bad odours.

TABLE III. Effect of antioxidants on sensory quality of fresh shucked oyster during storage at 1°C

treatment	days at 1°C		
	0	4	10
I	A 8.6a*	B 6.7	D 6.6c
II	A 8.6a	B 7.3b	C 5.8d
III	A 8.6a	A 8.4a	B 7.6b
IV	A 8.5a	A 8.2a	B 8.0a
V	A 8.6a	A 8.3a	B 8.2a
VI	A 8.4a	B 7.4b	D 3.6e
VII	A 8.3a	C 6.1c	D 2.6f

TABLE IV. Effect of antioxidants on sensory quality of frozen shucked oyster during storage at -20°C

treatment	months at -20°C			
	0	3	9	12
I	A 8.5a	B 6.0c	C 5.0b	D 4.5b
II	A 8.5a	B 8.2a	C 6.5a	C 6.5a
III	A 8.5a	A 8.0a	C 6.5a	D 6.3a
IV	A 8.5a	A 7.8ab	C 6.7a	D 6.5a
V	A 8.5a	A 7.5b	C 6.5a	D 6.5a
VI	A 8.3a	A 7.3b	C 6.5a	D 6.4a
VII	A 8.4a	A 7.4b	C 6.5a	D 6.3a

* Values in the same column bearing unlike letters differ significantly (P < 0.01)

The browning discoloration of oyster during refrigerated or frozen storage was believed to be due to the oxidation of lipid /4,12,19/. According to the present data, although the TBA value and acid value of samples treated with BHA remained almost constant (Figs 1 and 2), the degree of discoloration was severe and the hedonic scores were the lowest. However, the samples treated with water-soluble antioxidants kept their original colour during 4 days storage and only showed a slight discoloration after 10 days storage. It was clear that no relation between browning discoloration and the development of rancidity was observed from the data obtained in this experiment. According to some reports /20,21/, the oyster contains quite high levels of glycogen between post-spawning and next spawning. The free amino acids such as tyrosine were high in oyster meat /22/. In the same way as sugar and free amino acids affect the browning of oyster /18/, the addition of carbohydrates accelerated the occurrence of browning discoloration. It seems likely that the browning discoloration of oyster is probably due to the maillard reaction occurring during storage and thawing. The high level of glycogen breaks down readily during glycolysis after harvest and reacts gradually with the free amino acids contained in the oyster meat to produce the browning discoloration. It is also possible

that the water-soluble components such as tyrosine which was recognized to be high in oyster /22/ were oxidized in presence of polyphenolase and oxygen to form products which discoloured the meat.

Effect of the antioxidants and 3% sodium chloride immersion treatments on the prevention of browning discoloration and drip loss of frozen shucked oyster:

In experiment A), it is clear that the water-soluble antioxidants such as erythorbic acid, Na-erythorbate and Na-ascorbate showed good inhibitory effects on browning discoloration. Also, in a previous study /3/, immersion treatment with 3% sodium chloride before freezing indicated an excellent effect on reducing the drip loss from frozen shucked oyster. To understand the effect of the combined use of the antioxidants cited above and 3% NaCl on the prevention of drip loss and discoloration for long-term frozen storage of shucked oyster, the immersion treatments were performed. The results are shown in Figure 4 and Table IV. No distinct difference in the hedonic score (Table IV) among various antioxidants and 3% NaCl immersion treatments was observed after 9 months storage at -20°C . However, the hedonic score of the control samples was below 5 (not acceptable) after 12 months storage at -20°C , although samples treated with immersion treatments were still acceptable after 12 months storage. All samples treated with various antioxidants and 3% NaCl immersion treatment kept their original colour during 3 months storage. However, browning discoloration occurred after 9 months storage. The control group was discoloured slightly after 3 months storage and severely after 12 months storage. The effects of these immersion treatments on the reduction of drip loss upon thawing is shown in Figure 4. Clearly the drip loss of all samples treated with these immersion treatments was efficiently inhibited during a year's storage; the quantity of drip loss was decreased from 30% in control samples to about 14% in immersed samples after 12 months storage at -20°C .

This experiment showed that the immersion treatments could inhibit the drip loss from frozen oyster, but only a little effect on preventing the browning discoloration was observed. Perhaps the inhibitory effect on drip loss was due mainly to the penetration of sodium chloride and a subsequent increase in the water-holding capacity of the muscle /23/. However, the prevention of discoloration was considered to be due to the antioxidants which were coated around the surface of the oyster. Not much antioxidants adhered to the surface within only 15 minutes immersion treatment. In long-term frozen storage, dehydration on the surface always occurred because of sublimation, and discoloration resulting from oxidation occurred owing to the exposure of the oyster to the air.

Effect of the ice glaze treatments on the prevention of browning discoloration and drip loss:

Changes in drip loss and organoleptic quality of frozen oyster treated with ice glaze treatments are shown in Figure 5 and Table V. As shown in Table V, all the samples were still acceptable after 12 months storage at -20°C . All the treated samples still kept their original colour after 12 months storage, except that the treatment VI and VII revealed slight browning discoloration while the control sample was discoloured slightly after 3 months storage and became darker after 9 months storage. These data indicated that ice glaze treatments were effective techniques for preventing the browning discoloration during frozen storage.

From the Figure 5, it is obvious that no effect of the ice glaze treatments on reducing the drip loss was observed. The drip loss of all samples, including the control group, increased gradually during frozen storage. It was considered that, although the ice glaze solutions contained 3% sodium chloride, penetration of NaCl did not occur because the ice glaze solution was a frozen coating around the surface of oyster. Samples treated with ice glaze excluded oxygen during storage and thus prevented the discoloration.

TABLE V. Effect of ice glaze on the sensory quality of frozen shucked oyster during storage at -20°C

treatment	months at -20°C			
	0	3	9	12
I	A 8.4a*	B 6.0b	C 6.1c	C 5.8c
II	A 8.5a	A 8.4a	A 7.8a	A 6.8a
III	A 8.4a	A 8.3a	A 7.8a	A 7.1a
IV	A 8.4a	A 8.4a	A 7.4b	A 7.0a
V	A 8.4a	A 8.2a	A 7.4b	A 6.7a
VI	A 8.3a	A 8.2a	A 6.8c	A 6.2b
VII	A 8.4a	A 8.2a	A 7.9a	A 6.4b

TABLE VI. Effect of combined use of antioxidants and ice glaze on the sensory quality of shucked oyster during storage at -20°C

treatment	months at -20°C			
	0	3	9	12
I	A 8.5a	B 6.8c	C 6.2c	C 6.0c
II	A 8.5a	A 8.4a	A 7.6a	A 7.2a
III	A 8.5a	A 8.3a	A 7.8a	A 7.2a
IV	A 8.4a	A 8.1a	A 7.5ab	A 7.4a
V	A 8.5a	A 8.2a	A 7.8a	A 6.8b
VI	A 8.5a	A 8.1a	A 7.4b	A 7.1ab
VII	A 8.4a	A 7.8b	A 7.2b	B 6.8b

* refer to the footnote in Table III

Effect of the combined use of immersion treatment and ice glaze treatment on the prevention of browning discoloration and drip loss:

In the experiments mentioned above, the immersion treatments and ice glaze treatments showed excellent effects on preventing the drip loss and browning discoloration respectively. The effect of the combined use of these two treatments was investigated and shown in Table VI and Figure 6. As shown in Table VI, the treated samples were kept in good quality during 12 months storage at -20°C but the browning discoloration occurred in the control samples after 3 months storage and darkened progressively with prolonged storage.

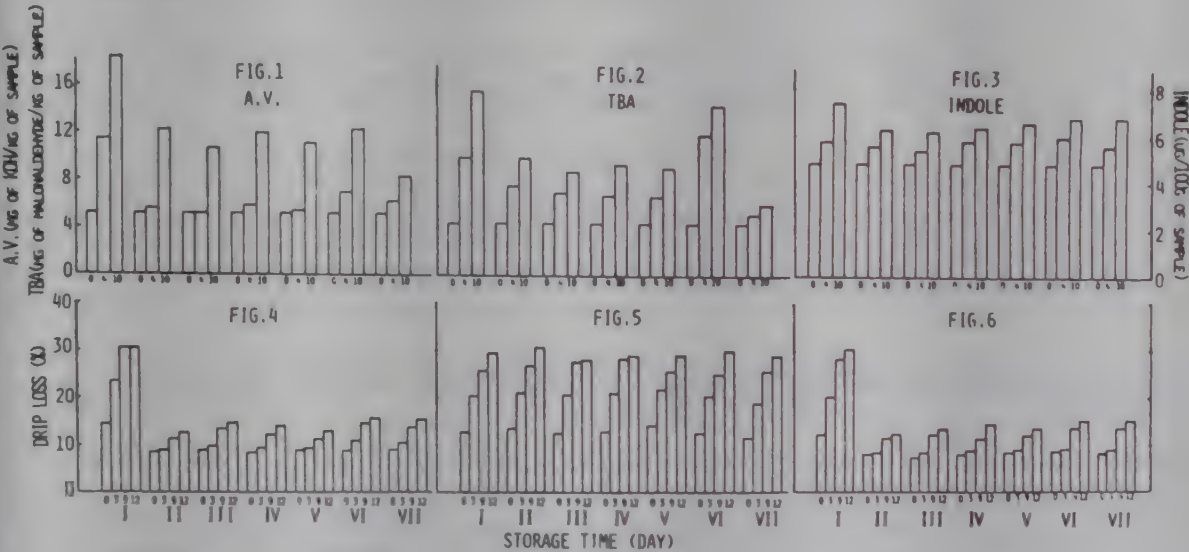


Figure 1. Effect of antioxidant immersion on the A.V. of shucked oyster meat during storage at 1°C
Figure 2. Effect of antioxidant immersion on TBA value of shucked oyster meat during storage at 1°C
Figure 3. Effect of antioxidant immersion on the indole content of shucked oyster meat during storage at 1°C
Figure 4. Effect of immersion treatments before freezing on the drip loss of frozen shucked oyster during storage at -20°C
Figure 5. Effect of the ice glaze treatment on the drip loss of frozen shucked oyster during storage at -20°C
Figure 6. Effect of combined use of immersion treatment and ice glaze treatment on drip loss of frozen shucked oyster during storage at -20°C

The effect of the combined use of these two treatments on reducing drip loss was shown in Figure 6. The quantity of drip loss of all treated samples was below 14% after 12 months storage at -20°C in stark contrast to that of the control group which was over 30%. It is evident that the combined use of immersion treatment and

ice glaze treatment were effective for the prevention of browning discoloration and reducing drip loss in frozen shucked oyster during long-term frozen storage.

REFERENCES

1. YAMASAKI, H.: The Materials of 6th Conference on the Utilization and Processing Studies of Marine Food in Japan. (1973), pp 258-269.
2. YAMASAKI, H.: *ibid.* (1975), pp 37-40.
3. JIANG, S.T., M.S. CHEN and C.H. WU: J. Fish. Soc. Taiwan 6(1):56 (1977).
4. YAMASAKI, H.: The Materials of 10th Conference on the Utilization and Processing Studies of Marine Food in Japan. (1976), pp 66-68.
5. MAHMOUD, T., SAUX, M-C, JOUZIER, E. and CROCKETT, R.: Annales de la Nutrition et de l'Alimentation 34(2):451 (1980), (from FSTA 2R194, 1981).
6. MORRE, J.: Bulletin de l'Academie Veterinaire de France. 52(2):319 (1979), (from FSTA 2R136, 1981).
7. BANKS, A., J.A. DASSOW, E.A. FEIGER, A.F. NOVAK, J.A. PETERS, J.W. SLAVIN and J.J. WATERMAN: In "Fundamentals of Food Freezing", Desrosier and Tressler ed., AVI Pub., (1977), pp 318-356.
8. WU, C.H., FUNG, K.K. and CHEN, M.S.: Bull. Taiwan Fish. Res. Inst. No 31, (1979), pp 332-344.
9. BOYD, N.S., WILSON, N.D.C. and HALL, B.I.: New Zealand J. of Sci. 23(2): 171 (1980).
10. TAKAGI, I. and SIMIZU, W.: Bull. Jap. Soc. Sci. Fish., 29:71 (1963).
11. HANSEN, P. and AAGAARD, J.: In "Freezing and Irradiation of Fish", Kreuzer ed., Fishing News (Books) Ltd. (1969), pp 147-158.
12. YAMASAKI, H.: Refrigeration, 49(561):619 (1974).
13. YAMASAKI, H. and YOSHIWA, T.: The Materials of the 8th Conference on Utilization and Processing Studies of Marine Foods in Japan (1974) pp 36-43.
14. YU, T-C and SINNHUBER, R.O.: J. Am. Oil Chem. Soc., 44:259 (1967)
15. SHINMA, I.: In "Suisan Seibutsukagaku Shyokuhin Zikensho", Saito *et al.* ed., Koseisha, Tokyo (1974), p 80.
17. AOAC: In "Official Methods of Analysis", 12th ed., AOAC, Washington, D.C. (1975), pp 317-318.
18. YAMASAKI, H., IYAMA, M., SUNAGAWA, M. and IMAI, H.: Kanzume Zihō, 44(3):39 (1965).
19. KUMATANI, Y.: In "Reitoshyokihin No Seizo", Koseisha, Tokyo (1974), pp 71-72.
20. GALTSOFF, P.S.: Fish. Bull. U.S. Dept Interior. 64:381 (1964)
21. JENG, S.S.: Bull. Inst. Zool., Academia Sinica, 18:1 (1979)
22. FUJII, M. and FUJIMARU, S.: Kanzume Zihō, 44(2):50 (1965)
23. CONNELL, J.J.: Symp. on Food: Protein and Their Reactions, Schultz ed., AVI (1964), pp 255-263.

PREVENTION CONTRE LE BRUNISSEMENT ET L'OXYDATION DES HUITRES ECAILLEES CONGELEES.

RESUME : Quelques traitements relativement simples et efficaces ont été mis au point en vue de protéger les huîtres écaillées congelées contre la décoloration et l'exsudation. Des huîtres fraîches, écaillées, ont été tout d'abord immergées à une température inférieure à 5°C pendant 15 minutes, dans une solution contenant 3 % de chlorure de sodium et 1 % des anti-oxydants suivants : erythorbate de sodium, ascorbate de sodium, acide érythorbique. Après égouttage et congélation rapide, des échantillons d'huîtres ont été glacés par immersion dans les mêmes solutions à raison de 4 à 5 % de leur poids. Aucun brunissement n'a été observé dans les échantillons traités après 12 mois d'entreposage, alors que ceux qui ne l'étaient pas présentaient un brunissement léger après 3 mois et sévère après 9 mois de stockage. L'exsudation a aussi été diminuée : 30 % dans les échantillons témoins contre moins de 14 % dans ceux traités, au bout de 12 mois à - 20°C.

H.R. KHANDAKER

Fisheries Development Corporation, Chittagang (Bangladesh)

M. HOLE AND C. JUKES

Humberside College of Higher Education, Grimsby (United Kingdom)

1. INTRODUCTION

For long term frozen storage of fish a temperature of -25 to -30°C is recommended and generally found acceptable for periods of over one year for lean fish if dehydration is controlled. For whole fatty fish stored in contact with air, a serious loss of quality can occur in less than 6 months due to oxidative rancidity (Ke et al/1/). Vacuum packaging in a good oxygen barrier film can extend the storage period for fatty fish to a year or more at -26°C (Ke et al/1/; Josephson et al/2/) and for at least 10 months at -18° (Lindsay/3/) whilst glazing alone at -29° prevents rancidity development in whole mackerel for at least 29 weeks (Hardy & Smith/4/). Dehydration can be controlled by glazing or by an inexpensive packaging film (eg polyethylene) and is a cost effective procedure but good oxygen barrier packaging films are expensive (Suggate/5/) - especially for a less developed country such as Bangladesh.

The present study was prompted by the availability of frozen storage capacity at -25 to -30° in Bangladesh and the seasonal abundance of River shad, a fatty fish (with up to 30% lipid) which is highly valued - ideally fresh and also after frozen storage, but not in cured form (Hasan/6/). River shad is caught over about a 5 month period and extending its availability throughout the year by frozen storage at -25 to -30° would be highly beneficial especially if it could be done without the need for an expensive packaging system. Mackerel (caught in UK waters) were used as representative fatty fish and the storage changes in both flavour and texture were assessed chemically and organoleptically at intervals over a period of 11 months.

Parallel storage trials were conducted on mackerel mince in order to assess more clearly the effect of the packaging systems, and also ascorbic acid, on rancidity development.

2. EXPERIMENTAL

Mackerel (*Scomber scombrus*) Fish were caught in the English Channel in the second week of December 1983 and iced immediately before despatch from Brixham to Grimsby. Torrymeter readings and assessment using the EEC sensory scheme indicated they had been in ice for 2 days but were Grade E (extra).

All fish were washed thoroughly in cold running water and some were filleted (skin on) for mince production using a Hobart mincer and Hobart mixer. Some whole fish were dipped in 4% ascorbic acid solution for 5 minutes and some fillets prior to mincing were dipped in 1% ascorbic acid for 30 seconds.

Freezing, packaging and storage. Whole fish and small blocks of mince were frozen down to -30° in a Jackstone laboratory horizontal plate freezer - freezing time was about $1\frac{1}{2}$ hours. The following plastic films, supplied by BXL Plastics Ltd., Darton, Barnsley were used for packaging of whole fish and mince:- (a) LDPE, low density poly-ethylene, 50 μm thickness, (b) LLDPE, linear low density polyethylene, 75 μm thickness, (c) Nylon laminate, 75 μm thickness, a coextrusion of LLDPE/Nylon LLDPE, (d) Eval laminate, ethylene vinyl alcohol laminate, 80 μm thickness, a coextrusion of LLDPE/Eval/LLDPE. LLDPE, Nylon laminate and Eval laminate were used for vacuum packaging (using a Multivac vacuum sealer) whilst LDPE was used to line jute bags (as used in Bangladesh) which were then simply stitched.

The following samples were stored at -25 to -30° in a chest freezer: (i) whole fish - vacuum packed, packed in LDPE-lined jute bags, and ice-glazed (but unpacked), (ii) ascorbic acid treated whole fish - ice glazed (but unpacked), (iii) fish mince, both untreated and ascorbic acid treated vacuum packed, and ice-glazed (but unpacked).

A second batch of mackerel was caught in the third week of September 1984 and treated as above.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

Properties of packaging films. Oxygen transmission rates at 23°C and 60% RH, and water vapour transmission rates, at 38°C and 90% RH, were measured using a Mocon Ox-Tran 100 Twin System and 'Dynamic WVTR Meter' respectively by J.E. Taylor at BXL Plastics, Ltd. Water vapour transmission rates were also measured using the calcium chloride dish method -BS 3177 (1959).

Analytical methods. Storage changes were measured at intervals of 25-40 days during the last 9-10 months of the 11 months storage of the first batch and for the first two months for the second batch. For whole fish the analyses were made on the fillets obtained after thawing. Total lipid was determined by a modified method of Bligh and Dyer/7/ using 2,6-ditertiary-butyl-p-cresol (BHT) in chloroform (0.01%).

The peroxide value (PV) of the extracted lipid was determined using a modified Lea method (Bligh & Dyer/7/) incorporating BHT in chloroform (0.01%) and storage in the dark for 30 minutes prior to titration with sodium thiosulphate. Thiobarbituric acid (TBA) values were measured on the fish flesh with skin using a modified distillation method of Sinnhuber and Yu (Tarladgis et al/8/) incorporating a BHA/BHT anti-oxidant mixture and distillation under nitrogen.

Soluble protein was measured on skinless flesh using the method of Cowie & Little/9/ and pH measurements were made on skinless fish muscle (Kelly et al/10/) after homogenisation with neutralised 0.005M iodoacetic acid. Water holding capacity (WHC) was measured as "centrifuge drip" of skin-on fish flesh (Del Valle and Gonzalez-Inigo/11/ and moisture content was determined at 105° (EEC recommended method-ISO R.1442-1973).

Sensory assessment. Whole fish after thawing were filleted, wrapped in aluminium foil and steamed for 20 minutes. Fresh fish (ie iced for 2 days) were similarly prepared. Trained panellists were asked to assess the samples according to the score sheet shown in Table I. Six samples, in two groups of three, were presented under white light to the 7 panellists. The mean was used to indicate the central tendency of the scores for each sample.

TABLE I
Taste Panel Score Sheet

Date:						
Code:						
CHARACTERISTICS	1	2	3	4	5	Score
Fish Odour	V.slight	Slight	Moderate	Strong	V.Strong	
Fish taste	V.slight	Slight	Moderate	Strong	V.Strong	
Rancid smell/taste	V.slight	Slight	Moderate	Strong	V.Strong	
Texture (mouth feel)	V.soft	Soft	Moderate	Sl.tough	Chewy stringy	

3. RESULTS

Packaging film properties. Table II compares the oxygen transmission rates (OTR) and water vapour transmission rates (WVTR) of the four films used.

TABLE II
Properties of packaging films

Property	LDPE	LLDPE	Nylon Laminate	Eval Laminate
O.T.R. ($\text{cm}^3 \text{m}^{-2} \text{day}^{-1}$)	4010	3020	43(35)*	0.4(0.55)*
W.V.T.R. ($\text{g m}^{-2} \text{day}^{-1}$)	12(13)#	8(8.9)#	7(7.5)#	5(2.6)#

* After storage at -30°C/5 months but measured at 23°C.

Calcium chloride method.

Clearly LDPE and LLDPE have much poorer oxygen barrier properties than Nylon or Eval laminates but the water barrier properties, whilst following the same trend, are much more similar. The oxygen barrier property of Nylon has been shown to increase as the temperature is lowered (Lindsay/3/).

Storage changes in mackerel mince Peroxide value (PV) and thiobarbituric acid value (TBA) changes during storage are shown in Table III. The maximum PV and TBA values obtained were significantly lowered both by ascorbic acid treatment and by vacuum packaging in a good oxygen barrier film such as Nylon or Eval laminate. For the PV changes distinct induction periods were shown for all but the untreated mince in Nylon and Eval laminates - these two showed values fluctuating with time and for which there was no significant ($p>0.05$) change with storage time.

TABLE III

Peroxide values and thiobarbituric acid values of untreated and ascorbic-acid treated mackerel mince stored at -25 to -30°C. Maximum values attained and time taken.

Packaging	Peroxide Values		Thiobarbituric Acid Values	
	Untreated	Treated	Untreated	Treated
Glazed-unpacked	66.8* (251)✓	20.7 (252)	23.2# (137)	10.4 (183)
LLDPE - vacuum	51.8 (251)	21.5 (216)	16.6 (230)	11.5 (264)
Nylon - vacuum	22.6 (251)	10.8 (292)	25.9 (137)	8.2 (183)
Eval - vacuum	13.1 (124)	11.8 (252)	18.6 (83)	4.4 (52)
	13.4 (251)		14.0 (83)	4.2 (82)

(*) Maximum peroxide value (mEq/kg lipid)

(✓) Time taken (days)

(#) Maximum thiobarbituric acid value (mg malonaldehyde/kg flesh)

Values are the mean of 3 determinations with coefficient of variations normally <5%. Initial values were PV=5.9 and TBA=3.6.

With regard to rancidity development, as indicated by PV and TBA changes, it was surprising how similar glazing without packaging and vacuum packaging in LLDPE were. Vacuum packaging in Eval laminate was superior in this respect to Nylon laminate in accord with their different oxygen barrier properties but this difference was very slight in the presence of ascorbic acid - especially as indicated by the TBA values where no significant increase occurred.

Storage changes in whole mackerel. Peroxide value (PV) and thiobarbituric acid value (TBA) changes are shown in Table IV.

TABLE IV

Peroxide value and thiobarbituric acid values for whole mackerel stored at -25 to 30°C. Maximum values attained and time taken.*

Packaging	Peroxide values		Thiobarbituric Acid values	
	Max. value	Time taken	Max. value	Time taken
Glazed - unpacked	20.0	194	14.2	96
	23.2 (19.6)#	293 (258)#	12.2 (8.1)#	265 (300)#
Jute bag-LDPE lined	25.6	293	9.2	161
			13.3	265
LLDPE - vacuum	21.6	293	8.4	161
			6.8	265
Nylon - vacuum	10.8	132	5.0	96
	11.9	258	5.2	300
Eval - vacuum	12.1	228	5.0	161

* See footnote to Table III

Treated with ascorbic acid

Each point is the mean of 3 determinations with coefficients of variation normally <3% for PV or <7% for TBA.

As for the stored mince, the good oxygen barrier films gave significantly lower maximum PV and TBA values. Simply glazing, or even packing (in air) in the stitched jute bag, was found to give similar PV values to vacuum packing in LLDPE although the TBA values were lower for the latter. Ascorbic acid treatment, prior to glazing, had a much smaller effect on the PV and TBA values than similar treatment of the mince. Compared to the mince, the PV and TBA values were significantly lower for the whole fish - except for the PV value for the Eval laminate. As found for the mince, the good oxygen barrier films were further characterised by showing PV's which fluctuated whilst the other systems had distinct maxima. For the TBA value changes, the situation was similar to the PV changes just described except that there were no significant changes ($p>0.05$) in TBA values for the Nylon and Eval laminates. In general terms the development of rancidity (as indicated by the PV and TBA values) falls into two groups (i) the good oxygen barrier films, Nylon and Eval laminates, and (ii) ice-glazing, LLDPE and the LDPE-lined jute bag.

Protein solubility changes are shown in Table V

TABLE V

Percentage soluble protein values for whole mackerel stored at -25 to -30°C

Packaging	Storage time (days)						
	0*	30	117	153	209	244	315
Glazed - unpacked	79	70 (73)#	72 (78)#	59 (72)#	63 (65)#	48	45
Jute Bag, LDPE-lined	79	69	71	66	65	56	49
LLDPE - vacuum	79	72	78	73	73	58	48
Nylon - vacuum	79	73	79	70	68	61	50
Eval - vacuum	79	74	74	68	72	57	52

*Fresh mackerel prior to freezing.

#Treated with ascorbic acid.

Each point is the mean of 3 determinations with coefficient of variation normally <4%

After a decrease associated with the freezing process, all the samples had reasonably constant values upto at least 120 days of frozen storage. After about 200 days all the samples showed a rapid decrease from 65-70% to 45-57% over the next 70 days, with a reduced rate of decrease to 45-52% at 315 days. In the period between 120-200 days, the vacuum packed samples (LLDPE, Nylon and Eval laminates) had reasonably constant values whilst the other samples were starting to decrease significantly - however, the overall changes were rather similar for all the samples.

pH Changes were monitored during the last 250 days of frozen storage and are shown in Table VI.

TABLE VI

pH Of mackerel flesh after storage as whole mackerel at -25 to -30°C

Packaging	Storage time (days)				
	55	145	197	246	302
Glazed unpacked	6.47	6.24	6.57	6.15	6.32
Jute bag, LDPE-lined	6.30	6.25	6.36	6.29	6.37
LLDPE - vacuum	6.34	6.18	6.44	6.21	6.23
Nylon - vacuum	6.46	6.18	6.40	6.22	6.22
Eval - vacuum	6.38	6.12	6.37	6.21	6.18

Each point is the mean of 6 determinations with coefficient of variation <1%.

No consistent trend was evident but generally pH values were lower relative to the value after 55 days storage. In general all samples had a minima after 80-150 days and again after 250-300 days. No correlation of pH changes with the packaging properties were evident - in fact the least fluctuation occurred for the LDPE-lined jute bag. Overall, however, there were no significant changes ($p > 0.05$) during the last 250 days of frozen storage for any of the packaging systems.

There were no significant ($p > 0.05$) changes in percentage water holding capacity (WHC) and moisture content during frozen storage for any of the packaging systems. From an initial WHC of 94.2% for fresh mackerel there was a drop on freezing to 88.5-89.7% and values of 85.5-91.8% after 100 days and 88.6-93.1% after 301 days for the range of packaging systems. Due to the variation in the lipid content (12-24%) and the associated change in moisture content which normally gives a combined lipid and moisture content of 80-85%, any dehydration occurring can be detected from changes in the combined lipid and moisture contents - since the lipid content is constant during frozen storage. From an initial value for fresh mackerel of 83.8% there was no change on freezing and values of 81.0-82.6% after 132 days and 82.5-84.8% after 294 days - indicating no significant dehydration (i.e. >5%) had occurred.

Sensory assessment. The mean scores for fish odour and taste and for rancidity and texture are compared for the different packaging systems in Table VII after 202 and 321 days of storage at -25 to -30°C.

TABLE VII

Taste panel results* for whole mackerel stored at -25 to -30°C

Packaging	202 days				321 days			
	Odour	Taste	Rancidity	Texture	Odour	Taste	Rancidity	Texture
Glazed-unpacked	2.0 (2.8)#	2.3 (2.7)#	1.2 (1.8)#	2.8 (2.7)#	1.9 (2.2)#	2.7 (2.7)#	1.6 (1.9)#	3.1 (2.9)#
Jute bag	2.2	2.7	1.5	2.7	2.2	2.5	1.3	3.6
LLDPE - vacuum	2.2	2.3	1.5	2.8	1.7	2.5	1.7	3.3
Nylon - vacuum	2.3	2.8	1.5	2.8	2.5	2.5	1.3	3.2
Eval - vacuum	2.2	2.7	1.8	3.0	2.2	2.6	1.4	2.9

* Means of 7 values with coefficients of variation from 45 to 70%.

Ascorbic acid treated

Values for fresh mackerel were:- odour 2.2; taste 2.7; rancidity 1.7 and texture 2.4.

Bearing in mind the format of the taste panel score sheet it was evident that (i) there was no significant ($p>0.05$) difference in organoleptic properties resulting from frozen storage in the different packaging systems, (ii) the samples stored for up to 321 days were not significantly different in rancidity, fish odour or fish taste from fresh mackerel. Only for texture were differences noted - with the stored samples being slightly firmer than the fresh mackerel.

4. DISCUSSION

Oxidative rancidity development should be retarded by restricting oxygen access, and its extent of development should relate to (a) peroxide formation and decomposition and (b) formation of carbonyl compounds as indicated by the PV and TBA values. Mince from fatty fish is especially susceptible to oxidative rancidity and for this system, packaging in films with a low oxygen transmission rate was shown to significantly reduce the development of oxidative rancidity as indicated by PV and TBA values (Table III). Further, the function of ascorbic acid as an oxygen scavenger and sequestrant correlates with the observed reduction in PV and TBA values and suggests that ascorbic acid treatment and vacuum packaging in Nylon or Eval laminate would be beneficial in retarding the development of oxidative rancidity. On the basis of PV and TBA values this conclusion is borne out with whole mackerel (Table IV) although the effect of ascorbic acid is much reduced. Vacuum packing in a poor oxygen barrier film such as LLDPE is no more effective than glazing or even the stitched, LDPE lined jute bags. The differences noted for whole mackerel on the basis of PV and TBA values were not found by the taste panel for the derived fillets steamed for 20 minutes. PV values of up to 26 were assessed as only very slight to slight rancidity. Other workers (Hardy & Smith/4/) have found great variation both in the actual PV's measured in similar systems and in relating absolute PV values with rancid odour - with values ranging from 4.8 to 30.6 being found for initial rancid odour detection. The PV is indicative of the potential source of carbonyl compounds (such as malonaldehyde measured by the TBA value) responsible for the rancid odour. In the good oxygen barrier films, it was noticeable that the TBA value dropped steadily on prolonged storage, especially with the mince, indicating an interaction, presumably with amino groupings, which could reduce the liberation of off-odours.

Changes towards a tougher texture can occur during frozen storage and often the change is greatest for lean or very fatty fish (Hanson and Olley 12). Additionally for lean fish, these texture changes are related with a decrease in the pH of the fish (cod) flesh for storage at -29°C (Cowie & Little/9/). Tables V and VI showed that whilst the decrease in soluble protein was large, and similar for all the packaging systems, the pH fluctuated during the last 250 days of frozen storage and, for example, was higher after 302 days than after 145 days (however, the difference was not statistically significant). The large changes in soluble protein were in contrast to the insignificant changes in water holding capacity and moisture content. Attempts to correlate texture changes, as determined organoleptically, with objective tests such as soluble protein, cell fragility (Kelly et al/10/) and pH for lean fish (especially cod) have led to the conclusion that only the pH relates to texture changes at very low temperatures. Additionally, it appears that it is the initial pH of the fresh lean fish (specifically cod - Kelly/13/), that relates to later texture changes rather than the actual pH at a particular storage time. Table VII showed that no significant change in texture was apparent during the period of rapid and large changes in the percentage soluble protein. After 321 days of storage at -25 to -30° all the samples were close to moderate texture with the sample stored in the stitched LDPE-lined jute bag being moderate to slightly tough. This contrasted with fresh mackerel which was assessed to have a soft texture - in fact the texture of the fresh mackerel appeared less acceptable than the stored samples.

It would appear that the temperature of storage and prevention of dehydration are most important at very low temperatures, and that whole mackerel can be maintained in an organoleptically acceptable condition for 11 months by storage at -25 to -30° in stitched, LDPE-lined jute bags.

5. ACKNOWLEDGEMENTS

General advice and test facilities for the packaging film measurements were provided by Dr. J.R.Suggate and Mr.J.E.Taylor of BXL Plastics Ltd Darton and Mr.D.Welham of Ross Foods, Grimsby.

REFERENCES

1. P.J.Ke, D.M. Nash and R.G. Ackman: Quality preservation in frozen mackerel. *Can. Inst. Fd. Sci. Tech.* 9 (1976) pp. 135-138
2. D.B.Josephson, R.C.Lindsay and D.A.Stuiber: Effect of handling & packaging on the quality of frozen whitefish. *J.Fd.Sci.* 50 (1985) pp.1-4.
3. R.C.Lindsay: The effect of film packaging on oxidative quality of fish during long-term frozen storage. *Fd.Prod.Dev.* 11, (1977), pp 93-96.
4. R.Hardy and J.G.M.Smith: The storage of mackerel (*Scomber scombrus*). Development of histamine and rancidity. *J.Sci.Fd.Agric.* 27 (1976) pp 595-599.
5. J.R.Suggate. BXL Plastics Ltd. Darton, Barnsley. Personal Communication (1984)
6. K.R.Hasan: Influence of packaging materials on the quality of frozen mackerel (*Scomber scombrus*) during storage. M.Phil Thesis, Humberside College of Higher Education (1984).
7. E.G.Bligh and W.J.Dyer: A rapid method of total lipid extraction and purification. *Can.J.Biochem. Physiol.* 37 (1959) pp 911-917.
8. B.G.Tarladgis, B.G.Watts, M.T.Younathan and L.R.Dugan: A distillation procedure for the quantitative determination of malonaldehyde in rancid foods. *J.Amer. Oil.Chem. Soc.* 37 (1960) pp 44-48.
9. W.P.Cowie and W.T.Little: The relationship between the toughness of cod stored at -29°C and its muscle protein solubility and pH. *J.Fd.Tech.* 1 (1966) pp 335-343.
- 10 K.Kelly, N.R.Jones, R.M.Love and J.Olley: Texture and pH in fish muscle related to cell fragility measurements. *J.Fd.Tech.* 1 (1966) pp.9-15.
- 11 F.R.Del Valle and J.L.Gonzalez-Inigo: A quick-salting process for fish. *Fd.Technol* 22 (1968) pp 1135 -
- 12 S.W.F.Hanson and J.Olley: Observations on the relationship between lipids and protein deterioration. In "The Technology of Fish Utilisation." Ed.R.Kreuzer, Fishing News (Books) Ltd., London, (1965) pp 111-115.
- 13 K.O. Kelly: Factors affecting the texture of frozen fish. In "Freezing & Irradiation of Fish" Ed.R.Kreuzer, Fishing News (Books) Ltd., London (1969) pp 339-342

PELLICULES D'EMBALLAGE POUR LA CONSERVATION DES POISSONS GRAS PAR CONGELATION - APPLICABILITE AU BANGLADESH.

RESUME : Des maquereaux entiers entreposés 11 mois à une température de - 25 à - 30°C en employant divers systèmes d'emballage ont été surveillés du point de vue de la transformation de la qualité en utilisant une série d'essais objectifs et organoleptiques. Les systèmes d'entreposage étaient les suivants : 1. glaçage, sans emballage, 2. trempage dans de l'acide ascorbique, glaçage, sans emballage, 3. emballage sous vide dans des pellicules souples en polyéthylène à basse densité linéaire (LLDPE) ou en laminé de Nylon ou d'Eval, 4. emballage dans des sacs de jute doublés de LDPE, tels que ceux utilisés au Bangladesh.

Pendant les 11 mois d'entreposage les essais objectifs ont fait apparaître des augmentations importantes des teneurs en peroxyde et en acide thiobarbiturique (ATB) mais celles-ci étaient réduites de façon importante avec les pellicules (laminés de Nylon et d'Eval), empêchant efficacement le passage d'oxygène. Les teneurs en protéines solubles ont diminué dans des proportions considérables (jusqu'à 45 à 50 %), tandis que le pH, la capacité de rétention d'eau et la teneur en humidité ne présentaient pas de modification, indépendamment des conditions d'entreposage à l'état congelé et des taux de transmission de vapeur d'eau des pellicules souples.

D'après les essais organoleptiques sur des maquereaux entiers, découpés en filets, cuits à la vapeur, il n'y avait pas de modification de l'odeur ni de la texture et pas de différences importantes dues aux différentes conditions d'entreposage.

On en conclut que les maquereaux entiers (et les poissons gras semblables) peuvent être maintenus dans un état organoleptique acceptable pendant au moins 11 mois en les entreposant à - 25, 30°C dans des sacs de jute doublés de LDPE, moyen d'emballage bon marché et facilement disponible au Bangladesh.

THE USE OF CYANOGEN BROMIDE TO STUDY PROTEIN CROSS-LINKING DURING FROZEN STORAGE
OF THE FLESH OF COD (*Gadus morhua*)

W.M. LAIRD and I.M. MACKIE
Ministry of Agriculture, Fisheries and Food, Torry Research Station,
135 Abbey Road, Aberdeen (United Kingdom)

1. INTRODUCTION

When the flesh of fish is held in the frozen state, the major contractile proteins suffer a loss in their water holding capacity. Depending upon the species and the conditions of storage, those changes can lead to an undesirable textural eating quality, to toughness and to a loss of the succulence characteristic of the unfrozen fish. While the rates of those deteriorative changes vary markedly from one species to another, the flesh of fish in general is less stable than that of other animals and it presents problems to the industry over loss of eating quality particularly when it is stored in the minced or comminuted form.

Despite considerable effort over many years to understand the toughening phenomenon resulting from frozen storage, the nature of the chemical interactions of the proteins remain largely unresolved /1/ /2/. It is established, however, that toughening is associated with a loss in extractability of the myofibrillar proteins in strong salt solutions and that most of the changes are believed to take place in myosin, the main component protein of the myofibrils. The sarcoplasmic proteins show little or no loss in extractability, but there is some evidence for a reduction in the activity of some of the enzymes in this fraction /1/.

It has been postulated that the toughening and loss of water holding capacity of the muscle is due simply to increased H- and ionic binding when the water environment of proteins is removed by freezing and frozen storage. Evidence now accumulating suggests that the process is more complex and that one or several types of covalent bond are also likely to be formed as, for example, -S-S-bonds, methylenic cross-linking formed by reaction of formaldehyde and primary amino groups or bonds formed by reaction of proteins and fatty acid hydroperoxides. Firm evidence identifying the formation of new covalent bonds during frozen storage deterioration is, however, lacking.

The approach in the present study has been to solubilise the salt inextractable residue without at the same time destroying evidence of the nature of the bonds which form on frozen storage. Cyanogen bromide is eminently suitable as it splits proteins only at methionine residues /3/ and should produce fragments of suitable size for examination by protein separation techniques of electrophoresis and HPLC. If covalent bonds are formed on frozen storage of flesh they should be detected as new peptides in the separation profiles. To eliminate differences which could arise from fish to fish variation and not from frozen storage, paired fillets from the same fish were stored for the same time (6 months) but at different temperatures -50°C and -15°C and -50°C and -6°C respectively. If differences could be detected by this approach they would be apparent in the portions stored at both -15°C and -6°C, periods of storage well beyond the times required for severe deterioration of fish flesh to take place.

2. EXPERIMENTAL

Materials

Fresh cod (*Gadus morhua*) was obtained from the Aberdeen market. Fillets were removed from the fish, frozen in an air blast freezer and sealed in polythene bags. One of each pair was stored at -50°C as a control and the other at -6°C or -15°C.

The chemicals used were either 'AR' Grade or specially prepared for High Performance Liquid Chromatography.

Methods

Preparation of cyanogen bromide peptides

(a) Removal of sarcoplasmic proteins

After storage for 6 months the fillets were removed from the cold stores and allowed to partially thaw at room temperature. A portion (25 g) of each fillet was cut into small pieces with a scalpel and suspended with stirring in 250 ml ice-cold 0.1M sodium chloride, 0.05 μ phosphate buffer, pH 7.5. After 1 h the supernatant solution was removed by filtration and the myofibrillar residue washed a further 3-4 times to obtain complete removal of the sarcoplasmic proteins.

(b) Reaction with cyanogen bromide

The washed residue was suspended in 400 ml, 80% formic acid solution. To a 50 ml portion, cyanogen bromide (3.0 g) was added and the reaction was then allowed to proceed for 2 days at room temperature. The excess of reagent and formic acid was then removed by evaporation to dryness in a rotary evaporator (Büchi). The peptide residue was then suspended in water (20 ml) and dialysed against a large excess of water to remove any remaining formic acid or cyanogen bromide. A soluble and an insoluble fraction were separated by centrifugation and freeze dried.

Isoelectric focusing on polyacrylamide gels

(a) Aqueous system

Commercially available pre-prepared polyacrylamide gels pH 3.5-9.5 were used. Isoelectric focusing was performed on 10 μ l aliquots of the peptide fractions (5.0 mg.ml⁻¹ in water) according to the manufacturers's instructions using a Multiphor 2117 (LKB Instruments Ltd).

(b) 6M Urea System

Gels containing 6M urea were prepared as follows:

A mixture of 3.33 ml, 29.1% w/v acrylamide solution, 3.33 ml 0.9 w/v N,N'-methylene bisacrylamide solution, 12.6 ml 10M urea, 1.27 ml ampholyte solution (pH 3.5-9.5) and 0.02 ml N,N,N',N'-tetramethylethylenediamine (TEMED) was deaerated for 5 min with a water pump. Ammonium persulphate solution, 0.45 ml, 3% w/v was then added carefully and, after mixing, the solution was introduced by capillary action into a 0.5 mm glass mould which had a sheet of Gelbond PAG (FMC Marine Colloids, USA) as one of its surfaces. The gels which formed were pre-run at 300 V for 20 minutes.

Samples of cyanogen bromide peptides (5.0 mg.ml⁻¹) for separation by isoelectric focusing were suspended in 6M urea and left at room temperature overnight. The water-soluble fractions dissolved readily but the water-insoluble samples did not dissolve fully.

Sodium dodecyl sulphate/polyacrylamide gel electrophoresis (S.D.S./PAGE)

Sodium dodecyl sulphate/polyacrylamide gel electrophoresis was performed in 6.5% acrylamide gels according to the method of Locker and Wild /4/.

High Performance Liquid Chromatography

(a) Reverse phase

A Vista 5500 liquid chromatography system (Varian) coupled to a Vista 402 control was used. Separations were made on a Hypersil WP-300, octyl column 5 μ (250 x 4.6 mm). The peptides were applied in 0.1% trifluoroacetic acid (TFA) and eluted with a gradient of 2-60% propan-2-ol in 0.1% TFA. The elution of the peptides was monitored at 225 nm.

(b) Size exclusion

A simple HPLC system incorporating a Rheodyne injection valve, a Waters M45 pump and Pharmacia UV-1 optical unit was used. The peptides were eluted iso-

cratically in either water or 6M urea through a TSK G3000 SW column (600 x 7.5 mm), fitted with a TSK SWP guard column (both supplied by Pharmacia), and the eluate monitored at 280 nm.

3. RESULTS AND DISCUSSION

It has been shown previously /2/ /5/ /6/ that fish flesh which has suffered extensive frozen storage deterioration can be dissolved in sodium dodecyl sulphate SDS solution even when the myofibrillar proteins are no longer extractable in salt solution. As SDS is known to be capable only of splitting H- and ionic bonds under the conditions of solution used, it has reasonably been inferred that frozen storage denaturation is due largely to the formation of non-covalent bonds. Electrophoresis in SDS, however, shows the presence of high molecular weight aggregates in solutions of denatured protein, presumably resulting from some forms of covalent bond formation, the extent and nature of which have yet to be determined.

The cyanogen bromide reaction

The initial step in the cyanogen bromide treatment involves the addition of the sarcoplasmic protein free, myofibrillar residue to 80% formic acid. The material from the flesh stored at -50°C dissolved readily but that from flesh stored at the two higher temperatures remained as a suspension and only dissolved after the addition of the cyanogen bromide. Differences between the low and high temperature stored flesh were again observed on removal of the formic acid and unreacted cyanogen bromide by dialysis when proportionally more precipitation took place in preparation from the latter samples (Table 1).

TABLE I

Dry matter distribution of CNBr peptides
from cod flesh held in frozen storage for 6 months

Storage Temp.	Water soluble (mg)	Water insol. (mg)	Total	Insol. as % total
-50°	160.6	1.3	161.9	0.8
- 6°	148.5	42.8	191.3	22.4
-50°	179.0	0.5	179.5	0.3
-15°	186.4	15.0	201.4	7.5

As any differences in properties or composition between the insoluble and soluble fractions may be significant, they were examined separately.

SDS Electrophoresis

SDS electrophoresis of the cyanogen bromide peptides (Fig 1a) produced the same distribution of molecular weights for all fractions. Comparison with the corresponding SDS electropherogram of untreated myofibrils (Fig 1b) shows the extensive breakdown to components almost as a continuum of molecular weights between $\approx 45,000$ and $\approx 10,000$. Distinct zones can also be identified against the background staining but there is no apparent relationship to frozen storage deterioration of the flesh itself, ie the absence of any new zones can be regarded as a lack of evidence for covalent bond formation. At the same time high molecular weight aggregates found in deteriorated flesh have been completely destroyed in the cyanogen bromide reaction.

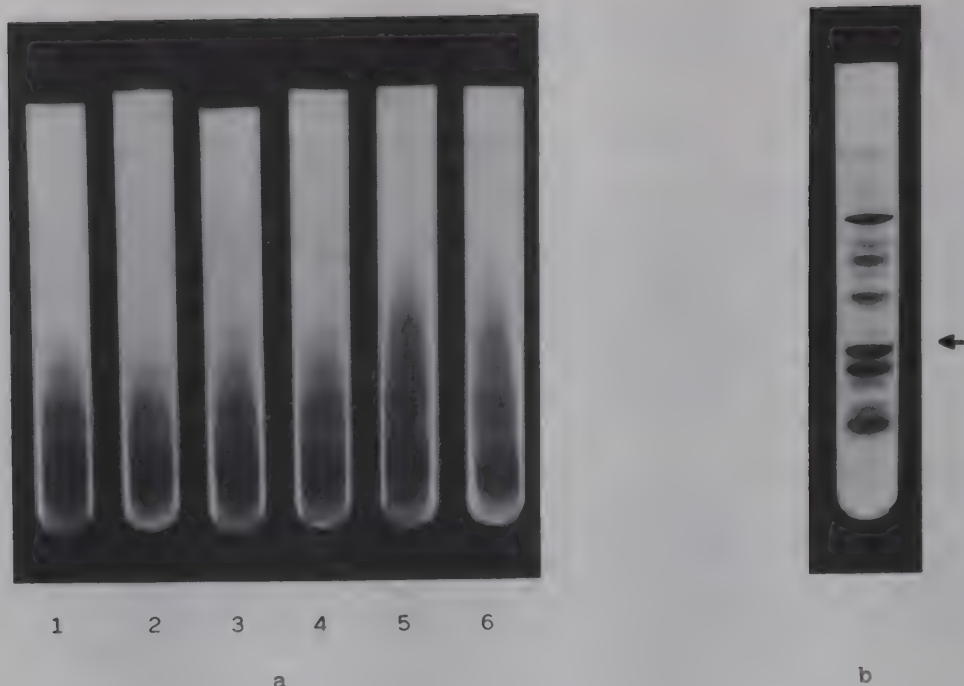


Fig. 1 - SDS/PAGE of cyanogen bromide peptides from cod flesh after 6 months frozen storage

1	-50°C	} water-soluble fractions
2	- 6°C	
3	-50°C	
4	-15°C	
5	- 6°C	} water-insoluble fractions
6	-15°C	

Untreated extract of
myofibrils

The arrow shows Actin
(M. Wt = 42,000)

Isoelectric focusing

Comparison of the water soluble fractions by isoelectric focusing in the standard urea-free system (Fig. 2a) appeared to show the disappearance of one protein zone (arrowed) from the separation profile of the -50°C stored fillets. As paired fillets have been used for these comparisons, the difference between the profiles can be attributed to the frozen storage history of the respective samples.

To allow comparison of the water-soluble with the water-insoluble peptides isoelectric focusing was carried out in 6M urea (Fig 2b) in which the latter peptides can be dissolved. The water-insoluble peptides differed from the water-soluble peptides in not having components with high isoelectric points but otherwise they seemed to be indistinguishable, although the former group showed additional zones close to the anode which other studies suggest may be due to carbamylation of amino groups on storage of the urea solutions. Again, comparison within the respective water-soluble and water-insoluble groups showed no differences.

Separation by HPLC

(a) Reverse phase HPLC

With recent developments in column efficiency and elution conditions of reverse phase chromatography it is now possible to separate peptides of the size obtained on reaction of myosin with cyanogen bromide. As the water-insoluble peptides could not be dissolved in a suitable solvent for reverse phase chromatography, only the water-soluble peptides were examined. The chromatogram obtained (Fig. 3a), using 0.1% trifluoroacetic acid (TFA) as solvent and a gradient of propan-2-ol in 0.1% TFA for elution of the peptides, showed no significant differences in the distribution of components between the three storage temperatures.

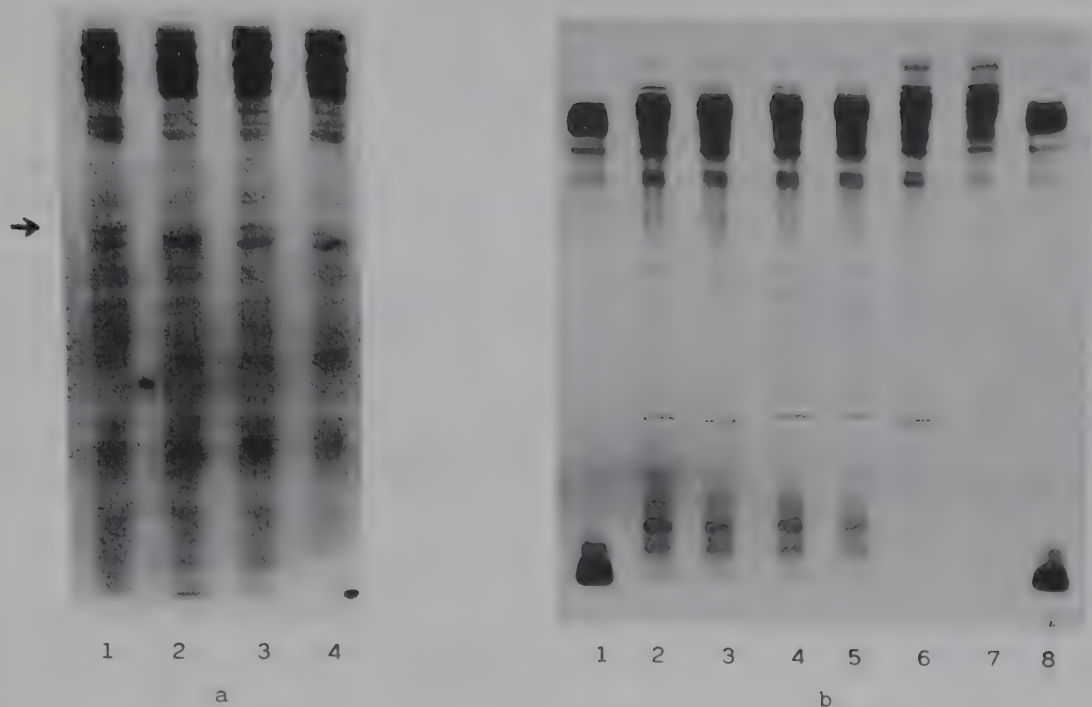


Fig. 2 - Isoelectric focusing of cyanogen bromide peptides from cod flesh after 6 months frozen storage

Water-soluble peptides

- 1 -50°C
- 2 - 6°C
- 3 -50°C
- 4 -15°C

1 and 8 are standard proteins

- 2 -50°C
- 3 - 6°C
- 4 -50°C
- 5 -15°C
- 6 - 6°C
- 7 -15°C

water-soluble peptides

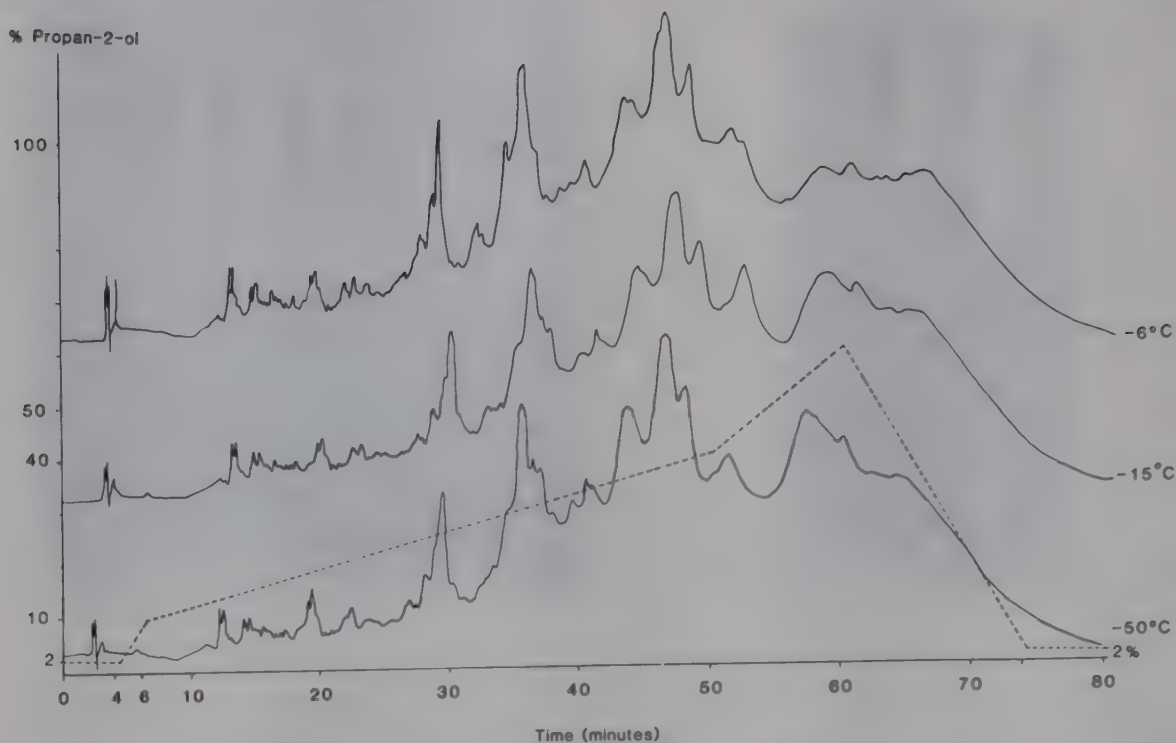
water-insoluble peptides

(b) Size exclusion

With 6M urea as solvent both the water-soluble and water-insoluble peptides could be compared under the same chromatographic conditions (Fig. 3b). The chromatograms for both groups of peptides from the -6°C stored fish are compared with the water soluble peptides for the -50°C stored fish and show a similar distribution of components between 45,000 and 11,500 molecular weights. The -6°C insoluble peptides, however, contain substantially more material at the exclusion limit of the gel than the corresponding water-soluble peptides for both -6°C and -50°C, indicative of new covalent bonds being formed on frozen storage.

4. GENERAL CONCLUSION

The analyses of the cyanogen bromide produced peptides from cod flesh stored for 6 months at -50°, -15° and -6°C have provided further evidence in support of the view that increased ionic and H-bonding take place on frozen storage deterioration. The formation of some degree of covalent bond formation also seems likely, but further investigations are required to determine the nature and extent of bonds formed. The significance of the proportion of water-insoluble cyanogen bromide peptides increasing with frozen storage deterioration needs further investigation, as does the presence of the apparent high molecular weight aggregate observed on size exclusion chromatography in 6M urea.



- a) Reversed phase chromatography of water soluble peptides loaded in 0.1% aqueous TFA and eluted with a gradient of propan-2-ol containing 0.1% TFA

- b) Size exclusion chromatography of water soluble and insoluble peptides.

Solvent - 6M urea in 0.05 M phosphate buffer (pH = 7.2)

The numbers indicate the elution volumes (ml)

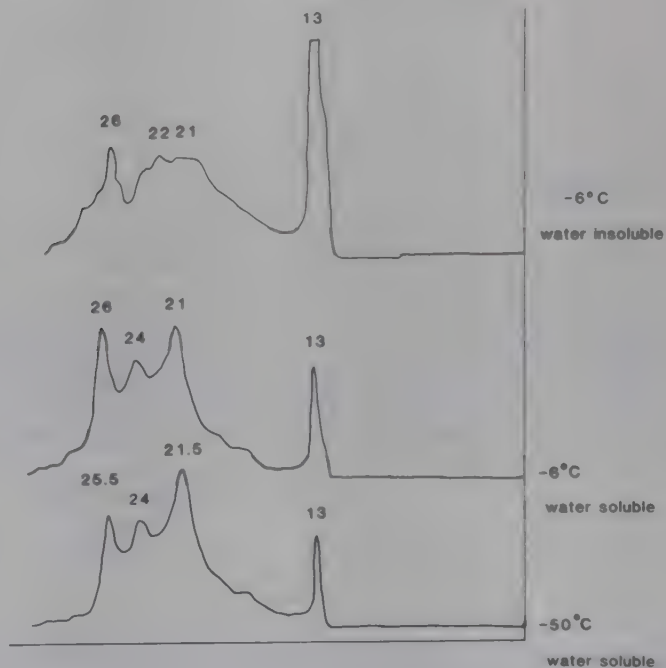


Fig. 3 - High Performance Liquid Chromatography

ACKNOWLEDGEMENT

The authors wish to acknowledge the technical assistance of Mrs Valerie Bond.

REFERENCES

1. J.J. MATSUMOTO : Chemical deterioration of muscle proteins during frozen storage. In Chemical Deterioration of Proteins (Eds J.R. Whitaker and M. Fujimaki) ACS Symposium Series 123, Washington, USA (1980) pp. 95-124.
2. W.M. LAIRD, I.M. MACKIE and T. HATTULA : Studies of the changes in the proteins of cod-frame minces during storage at -15°C . In Advances in Fish Science and Technology (Ed. J.J. Connell) Fishing News Books Ltd (1980) pp 428-434.
3. I.M. MACKIE : A review of some recent applications of electrophoresis and isoelectric focusing in the identification of species of fish in fish and fish products. In Advances in Fish Science and Technology (Ed. J.J. Connell) Fishing News Books (1980) pp. 444-450.
4. R.H. LOCKER and D.J.C. WILD. The fate of the large proteins of the myofibril during tenderising treatments. Meat Science 11 (1984) pp. 89-108.
5. H.K. LIM and N.F. HAARD : Protein insolubilization in frozen Greenland halibut (*Reinhardtius hippoglossoides*) J. Food Biochem. 8 (3) (1984) pp. 163-187
6. W.M. LAIRD and I.M. MACKIE : Protein changes during frozen storage of cod. I.I.R. - Commission C2 - Boston, USA (1981) pp. 349-355.

UTILISATION DU BROMURE DE CYANOGENE POUR L'ETUDE DES LIAISONS CROISEES DES PROTEINES DE LA CHAIR DE CABILLAUD (GADUS MORHUA) CONGELEE AU COURS DE SON ENTREPOSAGE.

RESUME : Le bromure de cyanogène, un agent spécifique de fractionnement des protéines, a été utilisé pour étudier la nature de l'interaction des protéines de la chair de poisson au cours de sa détérioration pendant l'entreposage à l'état congelé.

Des filets appariés de cabillaud ont été entreposés à -50 et -15°C et à -50 et -6°C , respectivement, pendant six mois, pour permettre le développement à la température la plus élevée d'une détérioration étendue pendant l'entreposage à l'état congelé. Les peptides produits après réaction avec le bromure de cyanogène ont alors été comparés par électrophorèse au sulfate dodécylique de sodium, par concentration iso-électrique et par chromatographie en phase liquide à haute pression. On n'a pas obtenu de preuve concluante de la formation de liaison covalente mais les résultats indiquent qu'il faut poursuivre les recherches sur les différences de répartition des peptides apparemment attribuables à la détérioration due à l'entreposage à l'état congelé.

FREEZING AND COLD STORAGE OF INDIAN FISH

P.A.PERIGREEN, JOSE JOSEPH and M.R.NAIR
Central Institute of Fisheries Technology
Cochin-682029, India

1. INTRODUCTION

The total fish landing in India is 2.3 million tonnes according to 1982 statistics. Crustaceans and cephalopods which constitute about 10% of the catch are mainly frozen for export. The quantity of other frozen seafoods exported from India is negligible. The Indian fish catch includes many commercially important species like oil sardine, mackerel, pomfrets, tuna, seer etc. The freezing and storage characteristics of many of the fishes have been studied in detail. This paper presents the results of these studies with particular emphasis on the frozen shelf-lives of Indian fishes.

2. RAW MATERIALS AND METHODS EMPLOYED

Small fishes like oil sardine (Sardinella longiceps), mackerel (Rastrelliger kanagurta), pomfrets (Stromateus cinereus, Pampus argenteus) and tilapia (Tilapia mosambica) are generally quick frozen either individually (IQF) or as blocks (BF) in contact plate freezers at -40°C /1,2,4,10,13/. These fishes are not usually gutted before freezing. Big fishes like tuna (Katsuwonus pelamis), spotted seer (Scomberomorus guttatus), cat fish (Tachysurus sp.) etc. are frozen as chunks, fillets or as gutted fish /5,6,7/. The fresh water fish like Labeo rohita, Catla catla, Cirrhina mrigala, Labeo calbasu, Mystus seenghala and Wallago attu are frozen as fillets/chunks in order to study the storage characteristics /8,9/.

The frozen fish are glazed in most cases and given a suitable packaging in order to protect the material from desiccation, oxidation and discolouration. Water is commonly used as glaze and in a few cases chemical glazes like 0.1% ascorbic acid or sodium chloride-glucose (0.5%) are used /10/. Anti-oxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and hydroquinone are also tried in fatty fishes /2/.

The fishes are usually stored in ice prior to freezing. The duration of pre-process iced storage varies from 0 to 14 days. In many cases uniced materials are used for freezing.

The storage temperature employed ranges from -10 to -23°C . The changes during frozen storage are assessed in most cases organoleptically and by following the changes in salt soluble nitrogen, alpha-amino nitrogen, non-protein nitrogen, total volatile bases and in the case of fatty fishes the changes in peroxide value, free fatty acids and thiobarbituric acid values are taken into consideration. The bacteriological changes are studied in a few cases /8,9/.

3. STORAGE LIVES OF FROZEN FISH

The details of freezing, storage and shelf-life of different Indian fishes are given in Table 1.

The freezing and storage characteristics of oil sardines, mackerel, pomfrets, tuna, cat fish and seer have been studied. Sardine is a fatty fish and fat content varies with seasons and maturity /2,4/. It is seen that there is an inverse relationship between the fat content of oil sardine and frozen shelf-life. Sardine with fat content 10% (dry weight basis) has a shelf-life of 20 weeks at -18°C and that with 35% fat (dry weight basis) has a shelf-life of only 10 weeks under the same conditions /2/. But it has also been observed that the shelf-life of frozen oil sardine is highly influenced by the initial quality of the raw material /4/. Rancidity development is a serious problem in the freezing preservation of oil sardine. Anti-oxidants like BHA and BHT are not effective to prevent fat oxidation in oil sardine. But dip treatment in 0.05% hydroquinone or a coating with agar agar is found to be effective /2/. A dip in 0.005% BHA for 15 minutes prior to freezing

increases the frozen shelf-life of pomfret (Pampus argenteus) /12/.

The white and red meat of tuna (Katsuwonus pelamis) behave differently during frozen storage; the red meat spoils more quickly compared to white meat. The shelf-life of frozen tuna also varies with the type of pack, that is, whole fish > chunk > fillets, the fillet being adversely affected during frozen storage /5/.

The studies on frozen storage of fillets from cat fish and spotted seer have shown that the IQF skin-on seer fillet wrapped in polythene has a shelf-life of 28-32 weeks at -23°C whereas the skinless fillets of cat fish wrapped in polythene have a shelf-life of 20 weeks at -18°C. The frozen shelf-life of seer fillets is reduced to 24 weeks by storing the fillets in ice for 3 days prior to freezing /6,7/.

The freezing and storage characteristics of a number of fresh water and brackish water fishes have been studied. (Table 1). In all the samples the decrease in salt soluble nitrogen and alpha-amino nitrogen coincides with a decrease in the organoleptic acceptability /8/. Tilapia (Tilapia mosambica) from fresh water and brackish water sources behave differently during iced and frozen storage. The frozen shelf-life of fresh water tilapia is not affected by storing in ice for 3 days prior to freezing while the shelf-life of frozen brackish water tilapia is reduced by 4 weeks by keeping 3 days in ice /13/. In the case of frozen whole milk fish (Chanos chanos) wide variations in frozen shelf-life (30 and 48 weeks) have been reported /14,15/.

4. FACTORS INFLUENCING SHELF-LIVES

The pre-process handling, glazing, packaging and storage temperature influence the frozen shelf-life of fish. Considerable reduction in frozen shelf-life has been observed in the case of oil sardine, tilapia, milk fish and seer fillets /1,13,14/ by storing in ice prior to freezing. Sardines stored in ice develop rancidity quickly and become unsuitable for freezing within 2-3 days in ice. Seven day iced milk fish has shelf-life of only 11 weeks compared to 30 weeks for fresh frozen fish /14/.

Table 1. Details of freezing, storage and shelf-lives of Indian fish

Name of fish	Pre-process condition, type of packing, freezing	Glazing	Storage temperature °C	Shelf-life weeks	Reference number
Oil sardine (<u>Sardinella longiceps</u>)	Fresh, whole IQF/BF	Water	-23	24	1
	One day iced, whole IQF/BF	"	-23	20	1
	Three days iced, whole IQF/BF	"	-23	12	1
"	Fresh, whole, IQF				
	(a) Fat 10% DWB	"	-18	20	2
	(b) Fat 22% DWB	"	-18	12	2
"	(c) Fat 35% DWB	"	-18	10	2
	Fresh, whole, BF				
	Fat 13% DWB	"	-15	15	3
Mackerel (<u>Rastreliger kanagurta</u>)	Fresh whole IQF	"	-15	16	10
	" " BF	"	-20	16	20
Tuna (<u>Katsuwonus pelamis</u>)	Fresh, gutted, whole	No glaze	-18	30	5
Spotted seer (<u>Scomberomorus guttatus</u>)	Fresh, skin-on		-10	16	6
	fillets, IQF	"	-23	32	

Cat fish (<u>Tachysurus</u> sp.)	Fresh skinless fillets, IQF	Water	-18	20	7
	Skinless fillets, BF	"	-18	27	7
<u>Labeo rohita</u>	Fresh fillets	-	-18	24	8
<u>Catla catla</u>	"	-	-18	20	8
<u>Cirrhina mrigala</u>	"	-	-18	24	8
<u>Labeo calbasu</u>	Fresh fillets	-	-18	24	8
<u>Mystus seenghala</u>	"	-	-18	20	8
<u>Wallago attu</u>	"	-	-18	20	8
White pomfret (<u>Stromateus</u> <u>cinereus</u>)	Fresh, whole IQF	Water	-15	14	10
	"	Ascorbic acid	-15	18	10
	"	NaCl- glucose	-15	22	10
White pomfret (<u>Pampus argenteus</u>)	Whole, IQF	BHA treated	-18	24	12
Surmai (<u>Cybium</u> <u>commersoni</u>)	Fresh, whole, IQF	Water	-15	16	10
	" "	Ascorbic acid	-15	20	10
<u>Tilapia</u> (<u>Tilapia</u> <u>mosambica</u>) fresh	Fresh, IQF	No glaze	-18	24	13
water	3 days iced IQF	"	-18	24	13
" brackish water	Fresh, IQF	"	-18	24	13
	3 days iced IQF	"	-18	20	13
Milk fish (<u>Chanos</u> <u>chanos</u>)	Fresh, whole IQF	Water	-18	30	14
"	Fresh, whole, IQF	-	-18	48	15
Bombay duck (<u>Harpodon</u> <u>nehereus</u>)	1 day iced, gutted, BF	No glaze	-23	12	16
Ribbon fish (<u>Trichiurus</u> sp.)	Fresh pieces, IQF/BF	"	-18	24	17
Shark (<u>Scoliodon</u> <u>laticudus</u>)	Fresh, gutted, beheaded	"	-18	28	18
Rawas (<u>Eleuthero-</u> <u>nema tetradactylus</u>)	Fresh, whole, IQF	"	-18	24	19

Note: DWB - Dry weight basis

The frozen shelf-life of IQF whole white pomfrets (Stromateus cinereus) is enhanced from 14 weeks to 18 and 22 weeks by using glazes like 0.1% ascorbic acid or sodium chloride-glucose (0.5% each) respectively /10/. Glaze helps to retain appearance, taste and quality over control during frozen storage /11/. The ascorbic acid glaze (0.1%) is also found to be effective in extending the shelf-life of IQF whole surmai (Cybium commersoni) from 16 weeks to 20 weeks at -15°C /10/. Better retention of soluble proteins is observed in glazed fish /21/. Glazing and proper packaging improved the shelf-life of frozen fillets. Frozen cat fish fillets stored without packaging developed dessication and discolouration after 5 weeks at -18°C. But the shelf-life of IQF fillets wrapped in 200 gauge polythene is about 20 weeks. Maximum shelf-life (27 weeks) is shown by fillets frozen as glazed blocks packed in polythene lined waxed cartons /7/. The effect of storage temperature on the quality and shelf-lives of some of the marine species (eg sardine, seer) has been documented. A comparative study of frozen storage of oil sardine (S. longiceps) at -12°C and -23°C reveals that the development of rancidity and denaturation are faster in samples stored at -12°C than that at -23°C /1/. The frozen shelf-life of fresh frozen fillets of spotted seer at -10°C and -23°C are found to be 16-20 weeks and 28-32 weeks respectively /6/.

5. CONCLUSION

In general, the frozen shelf-lives of many of the Indian fishes (fresh or one day iced) studied vary from 20 to 30 weeks at storage temperatures of -18 to -23°C which are commonly used in the fish processing establishments of the country. Fatty fishes showed relatively short shelf-life. Iced storage before freezing reduced the frozen shelf-life. Variation in shelf-life of the same species of fish has been reported by different workers. This may be due to the difference in the criteria adopted for the assessment of shelf-life (quality) or due to the difference in size, fishing ground, nutritional level of the water, ambient temperature, the method of catching and pre-process handling.

REFERENCES

1. A.V. SHENOY and V.K. PILLAI : Freezing characteristics of tropical fishes. 1 Indian Oil sardine. Fish. Technol. Vol.8 (1971) pp. 37-41
2. CYRIAC MATHEN, D.R. CHOWDHURI and V.K. PILLAI : Use of different glazes in frozen oil sardine. Fish. Technol. Vol.3 (1966) pp. 30-37
3. P.A. PERIGREEN, T.K. GOVINDAN and V.K. PILLAI : Effective method for reducing belly-bursting in frozen oil sardine. Fish. Technol. Vol.6 (1969) pp. 55-58
4. CHINNAMMA GEORGE, P.K. VIJAYAN and P.A. PERIGREEN : Utilization of frozen stored oil sardine for canning. Proceedings of the Symposium on Harvest and Post-harvest Technology of Fish, November, 1982, Cochin, India (in press)
5. CHINNAMMA GEORGE : Biochemical differences between the red and white meat of tuna and changes in quality during freezing and storage. Fish. Technol. Vol.12 (1975) pp. 70-74
6. A.V. SHENOY : Freezing characteristics of tropical fishes - iii - Spotted seer (Scomberomorus guttatus), Fish. Technol. Vol.13 (1976) pp. 105-110
7. P.A. PERIGREEN and JOSE JOSEPH : Freezing and cold storage of cat fish fillets. Fish. Technol. Vol.17 (1980) pp. 31-34
8. K. DEVADASAN, P.R.G. VARMA and R. VENKATARAMAN : Studies on frozen storage characteristics of fillets from six species of freshwater fishes. Fish. Technol. Vol.15 (1978) pp. 1-6
9. R. CHAKRABARTY : Changes in the muscle of three Indian major carps during frozen storage. Fish. Technol. Vol.21 (1984) pp. 91-93
10. M.G. JADHAV and N.G. MAGAR : Preservation of fish by freezing and glazing. Fish. Technol. Vol.7 (1970) pp. 146-149
11. S.S. PAWER and N.G. MAGAR : Chemical changes during frozen storage of Pomphrets, Mackerel and Sardines. J. Fd Sci. Vol.14 (1966) pp. 87-93
12. P.V. KAMASASTRI, S.N. DOKE and D. RAMANANDA RAO : Some aspects of freezing and frozen storage of pomfrets. Fish. Technol. Vol.4 (1967) pp. 78-84
13. A.V. SHENOY and M. ARUL JAMES : Freezing characteristics of tropical fishes - ii Tilapia. Fish. Technol. Vol.9 (1972) pp. 34-41
14. JOSE JOSEPH, P.A. PERIGREEN, C. GEORGE and T.K. GOVINDAN : Iced and frozen storage characteristics of cultured Chanos chanos. Fish. Technol. Vol.17 (1980) pp. 21-25
15. SIBSANKAR GUPTA, SUBRATA BASU, D. IMAM KHASIM and C.C. PANDURANGA RAO : Studies on preservation of cultured Chanos chanos by icing and freezing. Proceedings of the Symposium on Coastal Aquaculture, January 1980, Cochin, India (in press)
16. A.G. RADHAKRISHNAN, K.K. SOLANKI and R. VENKATARAMAN : Preliminary studies on freezing characteristics of Bombay duck. Fish. Technol. Vol.10 (1973) pp.124-130
17. R. BADONIA and K. DEVADASAN : Frozen storage characteristics of ribbon fish.

18. A.C. JOSEPH and K.K. SOLANKI : Frozen storage characteristics of shark (Scoliodon laticudus) Proceedings of the Symposium on Harvest and Post-harvest Technology of Fish, November, 1982, Cochin, India (in press)
19. D.K. GARG and JOSE STEPHEN : Frozen storage studies of rawas (Eleutheronema tetradactylus): Proceedings of the Symposium on Harvest and Post-harvest Technology of Fish, November, 1982, Cochin, India (in press)
20. A.N. BOSE : Freezing of tropical fish. In Freezing and Irradiation of Fish, Edited by R. KREUZER, (1969) pp. 179-188
21. P.L. SAWANT and N.G. MAGAR : Studies on frozen fish. I. Denaturation of proteins. J. Fd Sci. Vol.26 (1961) pp. 253-257

CONGELATION ET ENTREPOSAGE DU POISSON AUX INDES

RESUME : Le rapport passe en revue des études sur la congélation et les caractéristiques de l'entreposage frigorifique des poissons aux Indes. Des poissons pélagiques gras comme les sardines (Sardinella longiceps) et les maquereaux (Rastrelliger kanagurta) sont congelés individuellement ou en bloc de forme ronde et les plus gros sont congelés entiers, en gros morceaux ou en filets. La congélation est faite la plupart du temps en congélateur à plaque à - 40°C et la température d'entreposage est comprise entre - 10°C et - 23°C. Les poissons congelés sont glacés et la plupart du temps emballés pour les protéger des phénomènes d'oxydation, de dessiccation et de décoloration. Le glaçage est efficace pour accroître la durée de conservation à l'état congelé. On a aussi essayé les traitements anti-oxydants contre le rancissement pendant l'entreposage des poissons gras. Des variations saisonnières de la teneur en lipides agissent sur l'aptitude à conservation. La durée de conservation des filets congelés dépend également du type d'emballage autrement dit : poissons entiers > gros morceaux > filets. L'aptitude à la conservation des poissons congelés est appréciée par des indices chimiques et organoleptiques et, dans certains cas, on étudie aussi les modifications bactériologiques. Les A.A. discutent l'effet des procédés de préparation, de congélation, de glaçage, d'emballage et celui de la température de stockage sur la durée de conservation de nombre de poissons, commercialement importants, aux Indes.

THE FATE OF REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE IN MINCED FLESH
OF COD (*Gadus morhua*) AND ITS ASSOCIATION WITH FORMALDEHYDE PRODUCTION
DURING FROZEN STORAGE

P. REECE

Torry Research Station, Aberdeen (United Kingdom)

INTRODUCTION

It has been known for some time that formaldehyde and dimethylamine (DMA) are produced in the muscle of gadoid fish from the breakdown of trimethylamine-N-oxide (TMAO) by enzyme action during frozen storage /1/. It is now known that the enzymic breakdown of TMAO to DMA and formaldehyde requires reduced cofactors /2/. Both ascorbate-cysteine and reduced nicotinamide adenine dinucleotide (NADH)-flavin requiring enzyme systems have been identified in red hake muscle /2/, but the relative significance of these systems in cod muscle has not been determined. Previously reported data /3/ showed that the production of formaldehyde in minced cod muscle was inhibited by the presence of oxygen. Furthermore, the rate of production of formaldehyde during frozen storage of cod muscle mince fell with prior storage of the fish on ice. The rate of formaldehyde production was least if the cod had previously been stored on ice for about four days, a period which coincided with the time required to reduce NADH levels in cod muscle to a minimum /4/.

This paper therefore sets out to add further evidence for the role of NADH as an *in vivo* cofactor for formaldehyde production in minced cod muscle and to indicate a means of controlling formaldehyde production in mince by reducing the NADH content in the mince.

MATERIALS AND METHODS

1. Preparation of mince

The fish in these experiments were exclusively cod *Gadus morhua* which were processed immediately after catching or stored, gutted, on ice for four days before processing. Skinned fillets were passed through a Baader 694 flesh stripper with 5 mm diameter drum holes.

2. Storage of the mince

The fillet mince was well mixed and placed in aluminium foil dishes each 160 x 100 x 25 cm. The dishes were then frozen to -40°C in a horizontal plate freezer and the surface of the mince covered with a thin polythene film before the trays were wrapped in aluminium foil and stored at -10°C.

3. Determination of formaldehyde

At suitable intervals during the storage period, a dish of minced fish was removed and sliced, whilst still frozen, into 4 mm layers. Each layer was well mixed and then assayed for formaldehyde by the Nash reagent after distillation of the mince in acid solution as described previously /3/.

4. Determination of NADH

Frozen fish mince was differentially extracted and assayed for NADH by the method described by Jones and Murray /4/.

RESULTS

Measurements of the NADH and formaldehyde concentration in sections through blocks of cod mince during storage at -10°C showed that the NADH concentration fell most rapidly in the deeper layers of mince. This was associated with concentrations of formaldehyde which were much higher than at the surface (Fig. 1).

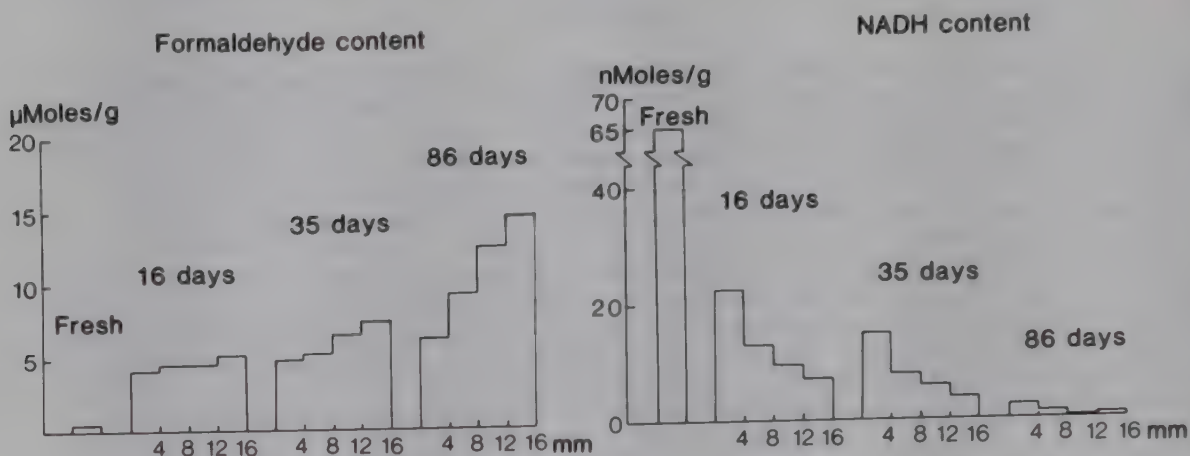


Fig. 1 - Effect of distance from the surface on formaldehyde and NADH content of fresh cod mince during storage at -10°C

Examination of NADH and formaldehyde produced in sections of frozen stored mince prepared from fish previously stored for four days on ice showed again that the NADH content fell most rapidly in the deeper anaerobic layers of mince and that it was associated with a more rapid rise in formaldehyde concentration. NADH levels were, however, significantly lower than in fresh fish mince and the associated rise in formaldehyde concentration was also much lower (Fig. 2).

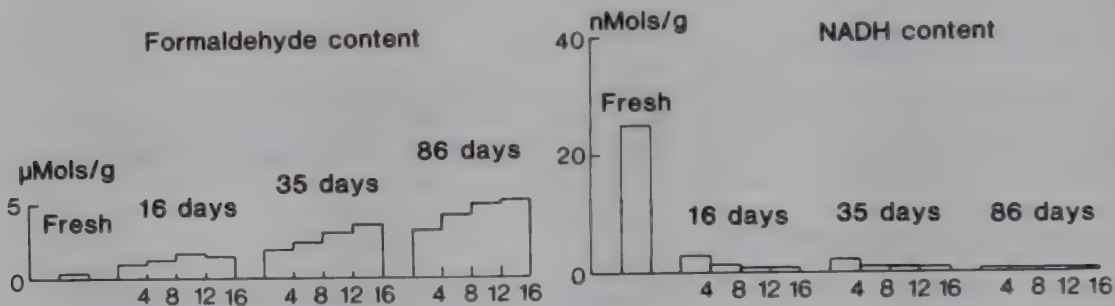


Fig. 2 - Effect of distance from the surface on formaldehyde and NADH content of cod stored 4 days on ice then minced and stored at -10°C

The marked fall in NADH content between the zero time and 16 days storage at -10°C , in both fresh and ice stored fish, was thought to be due to the oxidation of NADH by molecular oxygen trapped in the mince during preparation. The enzymic system responsible for the oxidation of NADH by molecular oxygen would have been released from subcellular particles by ice crystals produced during the freezing process and therefore would be available for catalysis after freezing. It was considered that such a procedure could be used to oxidise NADH in the fish muscle prior to frozen storage and hence reduce formaldehyde production.

Freezing followed by immediate thawing had been shown to rapidly eliminate 'freshness' fluorescence thought to be due to NADH (H K Davis, personal communication). Measurement of NADH concentration in fresh fillets processed through a freeze thaw cycle confirmed this observation (Table I).

TABLE I
Loss of NADH in fresh fish by freezing and thawing

	Fillet stored on ice 24 hrs	Paired fillet frozen then thawed over 24 hrs
	NADH content nMole g ⁻¹	NADH content nMole g ⁻¹
White muscle	2.5	1.0
Dark muscle	39.0	0.6

An examination of a freeze thaw procedure on formaldehyde production during frozen storage was therefore carried out. Cod mince was prepared from either cod stored on ice for one day or from cod frozen then thawed and stored on ice for a total processing time of one day. Measurements of formaldehyde and NADH concentrations through the depth of the mince during storage at -10°C showed that the NADH concentration after 16 days at -10°C was significantly lower in the mince produced from fish that had undergone a freeze-thaw cycle (Fig. 3). The treatment also resulted in a lower level of formaldehyde produced in the mince, especially in the deeper layers of mince. Formaldehyde concentrations did, however, continue to increase in the surface layers of mince from both treatments and approximately to the same extent.

DISCUSSION

The results presented suggest that removal of NADH from cod mince, by prior iced storage or by freezing and thawing, reduces the rate of production of formaldehyde in cod mince, especially in deeper layers, during storage at -10°C. Formaldehyde production does, however, continue slowly in the absence of NADH. This confirms earlier observations which showed that the oxygen sensitive formaldehyde production was inhibited by potassium cyanide but a residual oxygen insensitive production of formaldehyde persisted in the cod mince during frozen storage.

The determination of NADH and formaldehyde concentration in layers of cod mince showed that NADH consumption and formaldehyde production were not on an equimolar basis. Relatively much larger amounts of formaldehyde were produced than NADH consumed which suggests that either NADH is not directly involved in the production of formaldehyde in minced cod or that alternative sources of reduced cofactors are being used simultaneously.

Removal of NADH from the mince by freezing and thawing in the presence of oxygen clearly showed a marked reduction in subsequent rates of formaldehyde production during frozen storage. Similar observations were made by Hiltz *et al.* /5/ who showed that frozen silver hake immediately thawed, minced and refrozen produced less DMA than once frozen controls. This type of treatment, incorporating a short aerobic storage period after freezing and thawing, is to be preferred to the storage of minces under oxygen to inhibit formaldehyde production since oxidative changes in fish muscle, occurring during frozen storage, have been implicated in toughening /6/.

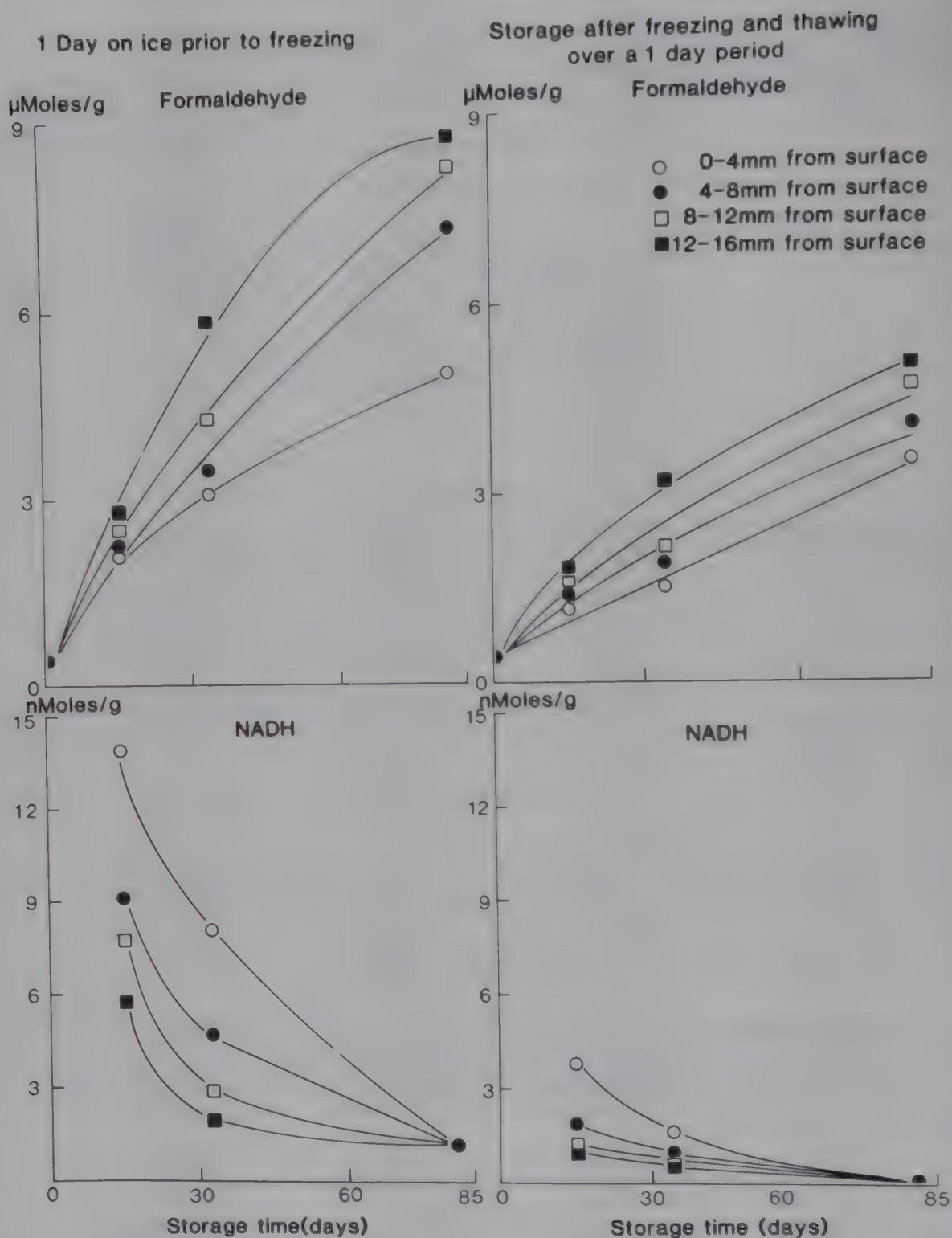


Fig. 3 - Effect of freeze-thaw treatment on subsequent rate of formaldehyde production and NADH content of cod mince during storage at -10°C

REFERENCES

1. K. YAMADA, K. HORADA and K. AMANO : Biological formation of formaldehyde and dimethylamine in fish and shellfish. VIII. Requirement of cofactor in enzyme system. Bull. Jap.Soc. Sci. Fish. (1969) 35 227-231.
2. M. BANDA and H. HULTIN : Role of cofactors in breakdown of TMAO in frozen red hake muscle. J. Food Process. & Preserv. (1983) 7, 221-236.

3. P. REECE : The role of oxygen in the production of formaldehyde in frozen minced cod muscle. J. Sci. Fd Agric. (1983) 34 1108-1112.
4. N.R. JONES and J. MURRAY : Nicotinamide adenine dinucleotide (NAD) and reduced NAD in living and chill stored dying muscle of cod. Bull. Jap. Soc. Sci. Fish. (1966) 32 2 197-203.
5. D.F. HILTZ, D.H. NORTH, B.S. LALL and R.A. KEITH : Storage life of refrozen silver hake processed as fillets and minced flesh from thawed, stored round-frozen fish. J. Fish. Res. Bd Can. (1977) 34 2369-2373.
6. C.H. CASTELL : Metal catalysed lipid oxidation and changes of proteins in fish. J. Am. Oil Chem. Soc. (1971) 48 645-649.

EVOLUTION DE LA NADH (NICOTINAMIDE ADENINE DINUCLEOTIDE REDUITE) DANS DU HACHIS DE CABILLAUD (GADUS MORHUA) ET SON ASSOCIATION AVEC LA PRODUCTION DE FORMALDEHYDE AU COURS DE L'ENTREPOSAGE A L'ETAT CONGELE.

RESUME : On sait que la production de formaldéhyde à partir d'oxyde de triméthylamine (TMAO) au cours de l'entreposage de muscle de gadoïdes exige d'autres facteurs réduits. La NADH-flavine et l'ascorbate-cystéine ont été impliqués dans cette réaction. La détermination de la formaldéhyde et de la NADH à travers l'épaisseur de muscle de cabillaud haché, au cours de l'entreposage à l'état congelé, a montré que la production de formaldéhyde coïncidait avec une perte de NADH, mais la production de formaldéhyde se poursuivait en l'absence de NADH. On a montré qu'un cycle de congélation-décongélation réduisait efficacement la concentration en NADH du muscle de cabillaud et aussi qu'il se produisait une concentration de formaldéhyde au cours de l'entreposage ultérieur à l'état congelé.

FROZEN FISH IN DISTRIBUTION IN THE UNITED KINGDOM

J. WIGNALL

Ministry of Agriculture, Fisheries and Food, Torry Research Station,
135 Abbey Road, Aberdeen (United Kingdom)

1. INTRODUCTION

The last major study of the frozen fish distribution system carried out by Torry staff was in 1971 /1/ and it was decided for a number of reasons to study the current situation. In the new study the range of products examined was extended to include vegetables and chickens as well as three types of fish products. The aims of the work were to establish the temperatures of storage prevailing, the times that food products are held in various stages in the distribution chain and the quality of the products at each stage.

It was not possible, in practical terms, to conduct the study on an ideal, statistically valid sampling regime because of the size of the frozen food industry and the limits on funds and staff available. Attempts were made, however, to ensure that sufficient data were collected in a series of detailed studies in order to make certain that a valid and reasonably representative picture of the distribution system was obtained.

2. OUTLINE OF THE STUDY

Products sampled

Samples of fish, vegetables and poultry were purchased, for detailed assessment, at all stages in the distribution chain. For this report, the remarks have been restricted to the fish products; the types of fish products sampled were (a) cod fillets/portions (uncoated FU), (b) cod fingers and portions (battered and breaded FB) and (c) cod in sauces (FS). When cod products were not available, haddock was substituted.

Primary cold stores

In this study primary cold stores are defined as stores holding commodities which have been processed into consumer packs ready to be delivered to secondary stores. In the main, the primary stores visited were sited near to the manufacturing or production units and served all the individual retail areas.

Secondary cold stores

Secondary cold stores are defined as stores from which goods, normally in wholesale packs, are delivered directly to retail outlets. In most cases the shops in each of the five areas were served by a separate group of secondary stores but, in some instances, the stores distributed goods nationally. Visits to the groups of stores were associated with each of the ten retail studies.

Retail shops

Five centres within the UK which represent different geographical types of distribution patterns and economic and social aspects of consumers were selected for the retail study /2/. Each area was visited twice at different times of the year with one exception. This area, Bath, was revisited approximately one year after the first visit. This pattern was chosen in order to be able to attempt an analysis of the results on the basis of regional and seasonal variation.

Four main types of retailers were studied in each area; hyper/super markets, stores belonging to national chains of food retailers, frozen food centres and small individual shops. The number of samples taken in each particular type of shop varied according to market share.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

3. MEASUREMENTS AND OBSERVATIONS

Temperature measurements

The within pack temperature of each sample was measured, as near as possible *in situ*, with a precooled temperature probe and the technique recommended by Codex Alimentarius was followed /3/. These packs were purchased and used for subsequent quality assessment. For the retail stage the position in the cabinet of each purchased pack was also noted. To supplement the within pack temperature measurement data, numerous non-destructive temperature measurements were made throughout the distribution chain by placing a temperature probe between packs or cases. In addition, air temperatures in the cabinets, cold stores and loading bays were measured.

Time in distribution

Assessments of time in the distribution chain were based on the rotation codes and the date codes used by individual firms. Full details were obtained from the samples purchased where possible and these data were supplemented by noting, at the time of each visit, codes on samples used for the non-destructive temperature measurements.

Quality assessment

Quality assessment of the fish was carried out by the staff of the Humber Laboratory within three weeks of the field work. Torry assessment schemes were used to obtain information on pre-freezing quality as well as changes which arise during freezing and cold storage /4/.

Miscellaneous information

Data on general observations made throughout the distribution chain were also recorded in order to obtain a more complete understanding of the situation; included in these were shop practices on receipt of goods, assessments of the extent to which cabinets were filled, types of cabinet etc. As far as the cold stores were concerned, the types of loading bays, methods of assembling loads and methods of stock control were noted.

Collection and transport of samples

Strenuous efforts were made to reduce to an absolute minimum any changes which might occur after sampling. The samples were collected in refrigerated insulated boxes and then placed, as soon as possible, in a refrigerated container held at -25°C . The samples were transported in this container to the Humber Laboratory for quality assessment, all subsequent storage was at -30°C .

4. SUMMARY OF THE RESULTS

Visits and sampling

The work was carried out over the period January 1982 to July 1983. The number of fish samples taken in each stage of the distribution network and the number of visits made to primary and secondary cold stores and retail premises are given in Table I.

TABLE I

Number of visits to cold stores and shops and
number of fish samples

Distribution stage	No. of visits to cold stores/shops	Number of samples			
		uncoated	coated	in sauce	total
Retail shops	219	397	440	270	1107
Secondary cold stores	66	185	189	123	497
Primary cold stores	24	80	113	39	232

Temperatures

Air temperatures in the cold stores and retail cabinets. The results of the air temperature measurements in the cold stores and retail cabinets are given in Table II. The mean air temperature in a cold store was obtained by taking the mean of temperature measurements made at nine positions spread evenly throughout the stores at ground level. For the retail cabinets, the air temperature quoted is the mean of temperatures taken at a number of accessible positions within the cabinet.

TABLE II

Air temperatures of the cold stores and
the retail cabinets

Temperature range °C	Primary stores Percentage in temperature range	Secondary stores	Retail cabinets
-29 to -24	61	23	11
-23 to -18	35	67	34
-17 to -12	4	10	37
-11 to -6	0	0	15
-5 and warmer	0	0	3
mean temperature	-23.9	-21.8	-16.7

The results show that the mean air temperature of the primary stores, the secondary stores and the retail cabinets are -23.9°C, -21.8°C and -16.7°C respectively, ie a rise in temperature between the primary stores and the retail cabinets of 7 degrees.

The modal temperature range for the primary stores was -29°C to -24°C whereas the modal range for the secondary stores was -23°C to -18°C. The percentages in the primary and the secondary stores in these ranges were 61 and 67 respectively. As one would expect, there was a greater spread of temperature in the retail cabinets, but 71% had air temperatures in the range -23°C and -12°C. However, in 18% of the cabinets, the air temperature was warmer than -12°C.

Product temperatures. The results of the product temperature measurements are given in Table III. It can be seen that the mean temperatures of all the fish products in the primary cold stores, the secondary cold stores and the retail cabinets were -21.5°C , -21.4°C and -16.0°C respectively. The temperatures of each product range within the individual stages were similar except the uncoated fish in the primary stores. The mean temperature of this product was -20.4°C which is significantly different from the coated fish samples, mean temperature -22.0°C and fish in sauce samples, mean temperature -22.4°C .

TABLE III
Temperature of fish in distribution

Temperature range $^{\circ}\text{C}$	Primary stores	Secondary stores	Retail cabinets
	Percentage samples in temperature range		
-30 and colder	0	0	0
-29 to -24	30	32	6
-23 to -18	58	54	33
-17 to -12	9	13	43
-11 to -6	1	1	16
-5 and warmer	1	0	2
Mean	-21.5	-21.4	-16.0

Examination of the product temperature distribution at each stage shows that there was little difference between the primary and the secondary stores with 88% of products in the primary stores and 86% of products in the secondary colder than -17°C . Considering products warmer than -12°C , there was 2% in the primary stores, 1% in the secondary stores and 18% in the retail stage. It should be noted that in the retail stage, the temperatures of packs above the load line were never taken. In fact, overloading was rarely observed and probably did not occur in more than 2% of all cabinets studied. However, the position in the cabinet influenced the temperature of the packs; the mean temperature of the packs in the top, the middle and the bottom layers were -13.4°C , -16.6°C and -17.9°C respectively, with 32% of the top layer packs warmer than -12°C .

Time in distribution

The cumulative time in distribution at each of the three stages is summarised in Table IV and shows a skew pattern. This suggests that the mean is not a satisfactory measure of the time in distribution because of the distortion caused by the long stay packs in the 'over 180 days' group and the median time is shown and this eliminates the distortion. The total mean time in distribution from packing to retail sale was 89 days with the median time of 68. In fact, 12% of the fish products was in distribution for 30 days or less and 64% for 90 days or less. The time in each stage was 60.5, 13.1 and 15.4 days for primary, secondary and retail storage respectively. Thus the products were held longest in the primary stores; the dwell times in the secondary stores and the retail shops were relatively short.

The time in distribution in the retail stage was affected by the position of the pack in the cabinet. In this study, it was shown that there was a tendency for the products in the bottom layer to be in distribution 11 days longer than those in the top layer. This trend, however, cannot be confirmed statistically because of the scatter of the results.

TABLE IV

Cumulative time in distribution of fish samples
at various stages

Time-Days	Primary stores	Secondary stores	Retail cabinets
	Percentage in the range		
1 to 30	52	27	12
31 to 60	19	33	31
61 to 90	9	16	21
91 to 120	7	6	17
121 to 150	4	7	7
151 to 180	3	6	3
over 180	6	5	9
Mean	60.5	73.6	89
Median	30	51	68

Quality assessment

Samples from the survey were presented to a trained taste panel which assessed the three major parameters, cooked flavour, cold storage flavour and toughness. The breaded and battered products have been grouped together but it must be remembered that this group is composed of a range of products such as battered fillets, battered portions from fillet blocks, fingers prepared from minced fish, as well as fillet blocks. The fish in sauce products were made from fillet blocks. Prior to cooking all the coatings were stripped off the breaded and battered samples and the sauce was removed from the fish in sauce samples.

Cooked flavour (freshness flavour) The taste panel results have been grouped into three categories: good, taste panel score 10 to 7; indifferent, taste panel score 6.9 to 5.5; and stale, taste panel scores less than 5.5. The results for the three product types at each distribution stage are given in Table V. It can be seen that there was little change in cooked flavour through the chain as anticipated. However, the battered/breaded products show a consistently higher proportion of samples in the middle range whereas no samples of fish in sauce had a score below 5.5. Few samples (not more than 4% in the uncoated and breaded/battered groups) were found to have marked negative characteristics such as strong off flavours, bitterness and sourness which suggests that reasonably fresh fish was used in the preparation of the products sampled.

TABLE V
Cooked flavour scores

Distribution stage	Uncoated fish			Breaded fish			Fish in sauce		
	Mean score	Percentage of samples		Mean score	Percentage of samples		Mean score	Percentage of samples	
		10 to 7	6.9 to 5.5		10 to 7	6.9 to 5.5		10 to 7	6.9 to 5.5
Primary cold stores	7.4	86	10	6.9	48	48	7.3	84	16
Secondary cold stores	7.5	81	18	7.0	52	46	7.4	82	18
Retail outlets	7.3	74	23	6.8	45	52	7.4	84	16

Cold storage flavour. This attribute of frozen fish has, like the cooked (freshness) flavour, been classified into three groups. The first group covers scores from 0 to 1.5 and represents fish with none or very little cold storage flavour development, the second group, scores 1.6 to 3.0, represents fish with moderate development of the flavour and the third group, scores greater than 3.1, reflect a strong and very easily detectable cold storage flavour. The results are shown in Table VI.

TABLE VI
Cold storage flavour

Distribution stage	Uncoated fish			Breaded fish			Fish in sauce		
	Mean score	Percentage of samples		Mean score	Percentage of samples		Mean score	Percentage of samples	
		0 to 1.5	1.6 to 3.0		0 to 1.5	1.6 to 3.0		0 to 1.5	1.6 to 3.0
Primary cold stores	1.1	74	26	1.3	64	35	1.1	76	24
Secondary cold stores	1.2	79	20	1.2	78	22	0.8	92	8
Retail outlets	1.5	61	34	1.5	59	38	1.1	80	19

Table VI shows that the mean cold storage flavour score tends to increase with the successive stages in the distribution chain, being highest at the retail outlet, as expected. Similarly, the proportion of samples exhibiting stronger flavours, although very small, tends to increase with successive stages in the distribution. The results tend to be less clear for the weaker flavours, however. Clearly, at both the primary and the secondary cold store stages, virtually all the samples were within the maximum limit of score 3 for cold storage flavour recommended in the Sea Fish Industry Authority (SFIA)/Torry Research Station Fish Purchase Specifications/4/. Only about 5% of the uncoated samples at the retail stage were considered to be above the recommended maximum; similarly, of the breaded/battered and fish in sauce

categories, only 3% and 1% respectively should be regarded as having strong cold storage flavours. At the other end of the scale 68%, 65% and 83% of all samples of uncoated fish, breaded/battered fish and fish in sauce respectively, had none or very little, cold storage flavour.

At the retail stage, the mean score for uncoated and breaded and battered products was no more than 1.5, whilst the fish in sauce scored 1.1. Thus there would appear to be no major problems overall with regard to this aspect of cold storage change. Although the differences between the product types were small, the lower score for cold storage flavour given by the panel for the fish in sauce products is statistically significant.

Toughness. The data from the survey have been divided into only two toughness categories, Table VII. Samples in the first group, scoring 2.0 or less on the toughness scale, exhibited the range of texture of unfrozen fish or only very mild toughening. The second group is composed of samples which showed definite signs of toughening.

TABLE VII
Toughening during cold storage

Distribution stage	Uncoated fish			Breaded fish			Fish in sauce		
	Mean score	Percentage of samples		Mean score	Percentage of samples		Mean score	Percentage of samples	
		-1.0 to 2.0	Above 2.0		-1.0 to 2.0	Above 2.0		-1.0 to 2.0	Above 2.0
Primary cold stores	1.6	73	27	1.6	73	27	1.8	60	40
Secondary cold stores	1.5	79	21	1.3	90	10	1.6	80	20
Retail outlets	1.7	72	28	1.4	85	15	1.9	62	38

One point stands out from the results given in Table VII; the majority of products were found to have textures within the normal range for unfrozen fish. more detailed analysis of the toughness data has shown that whilst fish in sauce products appeared tougher than uncoated and breaded products sampled in the retail outlets, cod in sauce was not significantly tougher than uncoated cod portions and steaks.

The mean toughness score for cod in sauce sampled in retail outlets was 1.9 (262 samples) and for uncoated cod steaks and portions 2.0 (102 samples). In contrast, uncoated fillets had a toughness score of 1.7 (158 samples) suggesting that products prepared from laminated blocks are tougher than uncoated fillets.

The percentage of samples within the normal toughness range for all three stages of the distribution chain is 73% for uncoated fish, 85% for breaded/battered and 67% for fish in sauce. However, the average taste panel scores are not very different. Overall, the differences between products are small but statistically significant.

5. GENERAL COMMENTS

It is clear from this survey that there was no observable change in intrinsic freshness during distribution. Fairly clear patterns of change have been demonstrated for cold storage flavour but the pattern is less clear for changes in toughness. On average, the cold storage changes are well within those that would be accepted in the SFIA/TRS purchase specifications. However, there are areas in which

improvement should be sought and, although the quality of the products was generally high, the industry should not be complacent, particularly in view of the percentage of products above -12°C in retail cabinets. The survey did not cover material prior to product manufacture or products stored by consumers.

ACKNOWLEDGEMENT

It was realised that if the objectives of the study were to be obtained, it could be necessary to obtain the complete agreement of all sections of the trade to gain access to their cold stores and cabinets to allow measurements and observations to be made on their premises and to supply details of date codes. We were most gratified to find that, with remarkably few exceptions, such permission was freely granted and that an excellent measure of cooperation was achieved with trade associations, companies and individuals throughout the industry.

REFERENCES

1. Study of the frozen fish distribution chain. Torrey Research Station (1971). Unpublished data.
2. Household Food Consumption and Expenditure 1978. Ministry of Agriculture, Fisheries and Food, National Food Survey Committee HMSO 1980.
3. Codex Alimentarius Commission. The recommended international code of practice for the processing and handling of quick frozen foods. Appendix I: Method of checking product temperature. Addendum I 1978 to CAC/RCP8 - 1976. Food and Agricultural Organisation of the United Nations: World Health Organisation.
4. Frozen Food in Distribution. Torrey Research Station 1983. Unpublished data.

LA DISTRIBUTION DU POISSON CONGELE AU ROYAUME-UNI

RESUME : Une étude sur la distribution du poisson congelé, au Royaume-Uni, a été effectuée de Janvier 1982 à Juillet 1983 inclusivement. L'étude a été divisée en trois stades : entreposage en magasin, en entrepôts de grossistes et en entrepôts de production. Pour une étude détaillée, cinq centres ont été sélectionnés dans cinq régions du pays parce que représentatifs de différents modes de distribution. Trois types de poisson ont été choisis et on a mesuré la température ainsi que la qualité aux différents stades de la distribution.

BIBLIOGRAPHY OF STORAGE LIVES OF WET AND FROZEN FISH

P. Howgate
MAFF, Torry Research Station, Aberdeen (United Kingdom)

1. SCOPE AND PURPOSE OF THE BIBLIOGRAPHY

This bibliography is a collection of references to publications where the authors have measured the quality of fish during storage by sensory methods and, on the basis of the results, have stated an estimate of the storage life of the product. The references are to the original work and do not contain any summaries or secondary publications quoting original work. Investigations in which quality was determined by non-sensory methods, for example extractable protein or lipid oxidation, are not included even though the authors make estimates of storage lives from the data.

The tabulations of the data, tables 1 and 2, draw attention to the sensory scales which were used to measure quality, their types and their lengths, and to the values on the scales which were used to define the ends of storage life. Some references in which the authors base their estimates of storage life on description of organoleptic properties alone have not been included.

It is not claimed that the bibliography is complete but it contains almost all the references within the scope just described, in publications readily available to fish technologists.

2. TABLES

Data extracted from the references are summarised in the tables. Tables 1 and 2 contain data on storage lives of wet fish and frozen fish respectively and on the sensory procedures used to determine them.

The columns in tables 1 and 2 are numbered and contain the following data:

Col 1	Reference
Col 2	Type of sensory scale coded as follows: Q Quality scale. One using pairs of terms like excellent/poor, good quality/bad quality, desirable/undesirable, acceptable/not acceptable. H Hedonic scale. Using the terms like/dislike. D Descriptive. The scale points are described in objective sensory terms like sweet, sour, bitter. R Rancidity. Intensity of rancid flavour.
Col 3	Range of the scale. The first value is that denoting the highest quality, the second the lowest quality.
Col 4	The value defining the end of storage life on the scale. In most references this value is explicitly stated by the authors. In others I have had to deduce the value from lines drawn on tables or figures or from internal evidence in the text, in this case the value is followed by ?. In a few cases the authors have used 2 values defining high quality and the limit of edibility.
Col 5	The common name of the fish under test. The specific name can be obtained from the cross references in table 3.
Col 6	The storage life in days in table 1 and in weeks in table 2. The mean value, rounded, is shown where a range of times are quoted in the reference.

- Col 7 The temperature, in degrees Celsius, or conditions of storage. In table 1 a numeric value denotes the product was stored in air at that temperature, otherwise storage is denoted as in ice or in refrigerated sea water (RSW).
- Col 8 Brief comments. Unless otherwise stated the storage time is for whole fish, gutted or ungutted as appropriate to the species or custom in the area in which the fish were caught. Special treatments intended to extend storage life are mentioned. Often various combinations of treatments were reported in the publication and a range of storage lives quoted. Where the conclusions are complex I have selected for inclusion in the table those treatments which were typical of the effects of the treatment or which were shown to be effective in extending storage life.

Table 3 is a cross tabulation of data to help in matching species with references.

3. DISCUSSION

All of the data on shelf lives in these references have been obtained using small panels, typically 5 - 10 members, of experienced assessors rating the changes in the stored products mostly on quality or acceptability scales. Only rarely have descriptive scales been used where the reader can relate his own experiences of testing stored fish products with those of the authors. It is not possible to compare results on quality scales since one group's ideas of what constitutes good - or poor - quality is not the same as another's; what is acceptable to one panel may not be acceptable to another.

Some investigators had their panels rate the fish on a hedonic scale but again with small numbers of experienced assessors. Liking or disliking of food products differs widely among individuals and hedonic tests should be applied to large numbers of assessors if the results are to be representative of some population. Apart from using only small numbers of assessors in the trials, the results cannot be considered as reflecting attitudes of consumers generally since perception of the quality of products on the part of experienced assessors must be influenced by their technical knowledge of the products and of the changes occurring during storage. In effect hedonic scales in the context of this bibliography are really quality scales and do not measure consumer acceptability.

Authors rarely define objectively the quality of the product at the end of the shelf life other than by the term describing the score at the limit. These are subjective terms such as just inedible, marginal quality, unacceptable, which are very dependent on the attitudes of the assessors. It is, therefore, not possible to compare estimates of shelf lives among the references on equivalent bases. However, it appears from experience at Torry Research Station of assessing spoiling fish of a wide range of species that the shelf lives listed in table 1 are the times to inedibility, not to the end of good quality.

In the case of only a very few species have storage lives been determined by more than one group of researchers and authors almost invariably have based their estimates of storage life on only one storage trial. Because of these factors it has to be accepted that the data are not very precise because they are not based on replicate determinations and are not accurate since individual panels are likely to be biased in comparison to a population of panels.

TABLE 1
Storage lives of wet fish

1	2	3	4	5	6	7	8
Ampola & Ronsivalli 1968	Q	5-1	2	haddock	12	ice	fillets
Amu & Disney 1973	Q	10-1	4?	bongo	21	ice	
				bumper	21	ice	
				burrito	21	ice	
				sea bream	21	ice	
Avdalov & Ripoll 1981	Q	10-0	4	Patagonian hake	15	ice	
Balakr.-Nair <u>et al</u> 1971	D	10-0	4	carp	35	ice	
Banik <u>et al</u> 1976	Q	10-0	5	chub mackerel white	11	ice	
				pomfret	27	ice	irradiated
Barassi <u>et al</u> 1981	Q	0-5	2.5	Sthn. blue whiting	6	ice	prespawning
					12	ice	postspawning
Barnett <u>et al</u> 1971	Q	10-1	5	rockfish	10	RSW	
					17	RSW	+ CO ₂
				chub salmon	11	RSW	
					>18	RSW	+ CO ₂
Barnett <u>et al</u> 1978	Q	10-1	5	pink shrimp	3	RSW	
					6	RSW	+ CO ₂
Barnett <u>et al</u> 1982	Q	5-1	2	salmon	>21	0	90% CO ₂
Beuchat <u>et al</u> 1973	Q	9-1	5?	channel catfish	14	ice	
Botta 1979	D	5-1	2	squid	10	ice	
Botta <u>et al</u> 1978	Q	5-1	2	capelin	12	ice	nonspawning
Botta & Shaw 1975	Q	6-1	3?	rougehead grenadier	13	ice	
Botta & Shaw 1976	Q	5-1	3?	round nose grenadier	18	ice	
Cheuk <u>et al</u> 1979	Q	9-1	6?	shrimp	10	ice	
			3?		16	ice	
Curran <u>et al</u> 1981a	Q	10-0	4	red snapper	32	ice	
				purple emperor	30	ice	
				Spanish mackerel	30	ice	
Curran <u>et al</u> 1980	Q	10-0	4	gold-lined sea bream	32	ice	
					8	10	
Curran <u>et al</u> 1981b	Q	10-0	4	threadfin bream	30	ice	
					12	5	
					7	10	
Dyer <u>et al</u> 1966	Q	100-0	40	swordfish	19	ice	
Emerson <u>et al</u> 1966	Q	10-1	5	yellow perch	8	0	fillets
					5	5	fillets
					55	0	irradiated
Fraser <u>et al</u> 1968	Q	100-0	40	mackerel	10	0	
					8	5	
					5	10	

1	2	3	4	5	6	7	8
Greig 1965	R	6-1	3	buffalofish	24	1	smoked
Hansen 1960	Q	10-0	4	plaice	16	ice	
				cod	13	ice	
Hansen 1963	Q	10-0	4	trout	10	ice	
Hansen 1972	Q	10-0	4	trout	9	0	
				herring	18	0	vac packed
					6	0	
					14	0	vac packed
Heaton <u>et al</u> 1972	Q	9-1	5	channel catfish	12	ice	
Huss 1972	Q	10-0	4	plaice	14	0	fillets
				haddock	18	0	vac pack
					13	0	fillets
					13	0	vac pack
Huss & Asenjo 1977	Q	10-0	4	Chilean hake	10	ice	
Huss <u>et al</u> 1974	Q	10-0	4	plaice	14	ice	
				cod	12	ice	
Jhaveri <u>et al</u> 1982	Q	9-1	4	mackerel	9	ice	
Kremsdorf <u>et al</u> 1979	H	5-1	3?	rat-tail	20	ice	
Liston <u>et al</u> 1961	D	5-1	2	Pacific rockfish	12	ice	
Lupin <u>et al</u> 1980	D	0-5	3.5	Patagonian hake	9	ice	summer
					14	ice	winter
Miyauchi <u>et al</u> 1964	D	10-0	5	king crab	5	0	cooked meat
					5	5	cooked meat
Ostavar <u>et al</u> 1967	Q	100-0	45	whitefish	22	0	
Partmann 1981	Q	9-1	6	trout	7	1	prepacked
					10	1	+ CO ₂
Patashnik 1966	Q	10-0	5	halibut	19	ice	
Peters <u>et al</u> 1963	Q	9-1	6	whiting (N. Amer.)	11	ice	
					11	RSW	
Poulter <u>et al</u> 1978	Q	10-1	4	chub mackerel /(a)	20	ice	
				grouper	30	ice	
				fusilier	16	ice	
Poulter <u>et al</u> 1981	Q	10-1	4	spotted batfish	22	ice	
				catfish	22	ice	
				spade fish	24	ice	
Reppond <u>et al</u> 1979	Q	5-1	3?	walleye pollock	6	ice	
					4	RSW	+ CO ₂
Ryder <u>et al</u> 1984	H	5-1	3?	jack mackerel (NZ)	7	ice	
Shaw & Botta 1975a	Q	100-0	40	capelin	9	ice	inshore male
Shaw & Botta 1975b	Q	100-0	40	capelin	2	RSW	inshore male
Shaw <u>et al</u> 1977	Q	100-0	40	witch flounder	18	ice	

1	2	3	4	5	6	7	8
Simmonds <u>et al</u> 1983	Q	10-0	7 5	Cape hake Cape hake	6 10	ice ice	
Sumner & Gorczyca 1981	D	5-1	2	flathead Japanese flounder whiting red snapper tarahiri Cape hake flathead Japanese flounder whiting red snapper tarahiri Cape hake	9 9 13 8 7 14 7 6 8 8 5 11	ice ice ice ice ice ice 5 5 5 5 5 5	
Teeny <u>et al</u> 1969	D	10-0	5	Dungeness crab	10	0	cooked meat
Varga <u>et al</u> 1974	Q	10-1	4.5	cod	10	0	fillets
Waters 1964	Q	5-1	3	calico scallops	12	ice	

TABLE 2

Storage lives of frozen fish

1	2	3	4	5	6	7	8
Andersson & Danielson 1961	Q	10-1	5	herring	8 48	-20 -20	fillets + ascorbic
Banks 1952	R	0-3	?	herring	26 16	-20 -20	whole fillets
Bosund & Garnot 1970	Q	9-1	5	herring cod	8 13	-15 -15	fillets fillets
Botta <u>et al</u> 1983	Q	5-1	2?	capelin	>91	-23	nonspawning
Crawford <u>et al</u> 1972	H	9-1	5?	Pacific hake	>52	-26	fillets
Dyer & Morton 1956	Q	100-0	40	plaice	26	-12	fillets
Dyer <u>et al</u> 1956	Q	100-0	75 50	rosefish	18 30 30 78	-12 -23 -12 -23	fillets fillets fillets fillets
Dyer <u>et al</u> 1966	Q	100-0	40	swordfish	1 20	- 4 -26	
Greig 1965	R	6-1	3	buffalofish	10	-20	smoked
Greig 1967a	R	5-1	3	chub	13 36	-20 -20	fillets + ascorbic
Greig 1967b	R	5-1	3	white bass	30 >56	-20 -20	+ ascorbic
Greig <u>et al</u> 1967	R	5-1	3	cisco	24 50	-18 -18	fillets + ascorbic
Gutschmidt & Partmann 1977	Q	9-1	6	saithe	22 30	-18 -24	fillets

1	2	3	4	5	6	7	8
Gutschmidt & Partmann 1977	Q	9-1		Saithe	42 >156	-30 -50	
			5	Saithe	34 56 78 >156	-18 -24 -30 -50	
Huss & Asenjo 1977	Q	10-0	4	Chilean hake	>26 >26	-15 -25	
Körner & Schreiber 1980	Q	9-1	6	marble perch	52	-28	fillets
				antarctic gurnard	52	-28	fillets
				black hake	78	-28	fillets
Lauder <u>et al</u> 1970	Q	100-0	40	redfish	>0	-23	
Licciardello <u>et al</u> 1980	Q	9-1	6	Argentine hake	10	-18	
Licciardello <u>et al</u> 1980	Q	9-1	6	Argentine hake	22 38 44	-18 -18 -18	+ erythrobrate saberised
Licciardello <u>et al</u> 1982 a	R	5-1	3	whiting	4	- 7	mince block
Licciardello <u>et al</u> 1982 b	Q	5-1	3	red hake	3 7 25 71 150	- 7 -12 -15 -20 -29	
Liljemark 1964	H	10-1	6	herring mackerel	8 8	-20 -20	
MacCallum <u>et al</u> 1966	Q	100-0	40	cod	40	-23	
Miyauchi <u>et al</u> 1975	R	5-1	3	black rockfish	17	-18	mince
Partmann 1981	Q	9-1	6	trout	12	-12	prepacked
Peters <u>et al</u> 1963	Q	9-1	6	whiting (N. Amer.)	10	-18	
Peters <u>et al</u> 1968	Q	9-1	6?	cod	>52	-18	
Poulter 1978	Q	10-1	4	chub mackerel	17 >52	-10 -30	
Rivero 1981	Q	10-0	4	Argentine hake	52	-19	
Shaw & Botta 1977	Q	100-0	40	capelin	>104	-23	inshore male
Stansby & Dassow 1949	R	5-1	3	rockfish	14 56	-18 -18	saberised

TABLE 3

Common names, specific names and references

Common Name	Specific Name	Reference
Antarctic gurnard	<u>Notothenia gibberifrons</u>	Korner & Schreiber 1980
Argentine hake (see also Patagonian hake)	<u>Merluccius hubbsi</u>	Licciardello <u>et al</u> 1980 Rivero 1981
Barred Spanish mackerel	<u>Scomberomorus commersoni</u>	Curran <u>et al</u> 1981a
Black hake	<u>Dissostichus eleginoides</u>	Korner & Schreiber 1980
Black rockfish	<u>Sebastes</u> spp	Miyauchi <u>et al</u> 1975
Bongo	<u>Ethmalosa dorsalis</u>	Amu & Disney 1973
Buffalofish	<u>Ictiobus cyprinellus</u>	Greig 1965
Bumper	<u>Chloroscromus chrysarus</u>	Amu & Disney 1973
Burrito	<u>Brachydeuterus auritus</u>	Amu & Disney 1973
Calico scallops	<u>Pecten gibbus</u>	Waters 1964
Cape hake	<u>Merluccius capensis</u>	Simmonds <u>et al</u> 1983 Sumner & Gorczyca 1981
Capelin	<u>Mallotus villosus</u>	Botta <u>et al</u> 1983 Botta <u>et al</u> 1978 Shaw & Botta 1975a and b, 1977
Carp	<u>Cirrinha mrigala</u>	Balakrishnan-Nair <u>et al</u> 1971
Catfish	<u>Netuma serratus</u>	Poulter <u>et al</u> 1981
Channel catfish	<u>Ictalurus punctatus</u>	Beuchat <u>et al</u> 1973 Heaton <u>et al</u> 1972
Chilean hake	<u>Merluccius gayi</u>	Huss & Asenjo 1977
Chub	<u>Leucichthys hoyi</u>	Greig 1967a
Chub mackerel(a)	<u>Rastrelliger brachysoma</u>	Poulter 1978 Poulter <u>et al</u> 1978
Chub mackerel(b)	<u>Rastrelliger kanagurta</u>	Banik <u>et al</u> 1976
Chub salmon	<u>Oncorhynchus keta</u>	Barnett <u>et al</u> 1971
Cisco	<u>Coregonus artedi</u>	Greig <u>et al</u> 1967
Cod	<u>Gadus morhua</u>	Bosund & Garnot 1970 Hansen 1960 Huss 1974 MacCallum <u>et al</u> 1966 Peters <u>et al</u> 1968 Varga <u>et al</u> 1974
Dungeness crab	<u>Cancer magister</u>	Teeny <u>et al</u> 1969
Flathead	<u>Neoplatycephalus richardsoni</u>	Sumner & Gorczyca 1981
Fusilier	<u>Caesio cuning</u>	Poulter <u>et al</u> 1978

Common Name	Specific Name	References
Gold lined sea bream	<u>Rhabdosargus sarba</u>	Curran <u>et al</u> 1980
Grouper	<u>Plectoma mocolatum</u>	Poulter <u>et al</u> 1978
Haddock	<u>Melanogrammus aeglefinus</u>	Ampola & Ronsivalli 1968 Huss 1972
Halibut	<u>Hippoglossus hipoglossus</u>	Patashnik 1966
Herring	<u>Clupea harengus</u>	Andersson & Danielson 1961 Banks 1952 Bosund & Garnot 1970 Hansen 1972 Liljemark 1964
Jack mackerel (NZ)	<u>Trachurus novaezelandiae</u>	Ryder <u>et al</u> 1984
Japanese flounder	<u>Rhombus maximus</u>	Sumner & Gorczyca 1981
King crab	<u>Paralithodes camtschatica</u>	Miyauchi <u>et al</u> 1964
Mackerel	<u>Scomber scombrus</u>	Fraser <u>et al</u> 1968 Jhaveri <u>et al</u> 1982 Liljemark 1964
Marble perch	<u>Notothenia rossi marmorata</u>	Korner & Schreiber 1980
Pacific hake	<u>Merluccius productus</u>	Crawford <u>et al</u> 1972
Pacific rockfish	<u>Sebastes</u> spp.	Liston <u>et al</u> 1961
Patagonian hake (see also Argentine hake)	<u>Merluccius hubbsi</u>	Avdalov & Ripoll 1981 Lupin <u>et al</u> 1980
Pink shrimp	<u>Pandalus</u> spp.	Barnett <u>et al</u> 1978
Plaice	<u>Pleuronectes platessa</u>	Dyer & Morton 1956 Hansen 1960 Huss 1972 Huss <u>et al</u> 1974
Purple emperor	<u>Lethrinus lentjan</u>	Curran <u>et al</u> 1981a
Rat-tail	<u>Coryphaenoides acrolepis</u>	Kremsdorf <u>et al</u> 1979
Red hake	<u>Urophycis chuss</u>	Licciardello <u>et al</u> 1982b
Red snapper	<u>Lutjanus sanguineus</u>	Curran <u>et al</u> 1981a
Red snapper	<u>Trachichthodes gerrardii</u>	Sumner & Gorczyca 1981
Redfish	<u>Sebastes marinus mentella</u>	Lauder <u>et al</u> 1970
Rockfish	<u>Sebastes flavidus</u>	Barnett <u>et al</u> 1971 Stansby & Dassow 1949
Rosefish	<u>Sebastes marinus</u>	Dyer <u>et al</u> 1956
Roughead grenadier	<u>Macrourus berglax</u>	Botta & Shaw 1975
Roundnose grenadier	<u>Coryphaenoides rupestris</u>	Botta & Shaw 1976
Saithe	<u>Gadus virens</u>	Gutschmidt & Partmann 1977

Common Name	Specific Name	References
Salmon	<u>Oncorhynchus</u> spp.	Barnett <u>et al</u> 1982
Sea bream	<u>Pagelus</u> <u>caupei</u>	Amu & Disney 1973
Shrimp	<u>Penaeus</u> spp.	Cheuk <u>et al</u> 1979
Southern blue whiting	<u>Micromesistius</u> <u>australis</u>	Barassi <u>et al</u> 1981
Spade fish	<u>Ephippus</u> <u>orbis</u>	Poulter <u>et al</u> 1981
Spotter bat fish	<u>Drepara</u> <u>punctata</u>	Poulter <u>et al</u> 1981
Squid	<u>Illex</u> <u>illecebrosus</u>	Botta 1979
Swordfish	<u>Xiphias</u> <u>gladius</u>	Dyer <u>et al</u> 1966
Tarahiri	<u>Nemadictalus</u> <u>macroptens</u>	Sumner & Gorczyca 1981
Threadfin bream	<u>Nemipterus</u> <u>japonicus</u>	Curran <u>et al</u> 1981b
Trout	<u>Salmo</u> <u>gairdneri</u>	Hansen 1963 Hansen 1972 Partmann 1981
Walleye pollock	<u>Theragra</u> <u>chalcogramma</u>	Reppond <u>et al</u> 1979
White bass	<u>Roccus</u> <u>chrysops</u>	Greig 1967b
White pomfret	<u>Pampus</u> <u>argenteus</u>	Banik <u>et al</u> 1976
Whitefish	<u>Coregonus</u> <u>clupeaformis</u>	Ostavar <u>et al</u> 1967
Whiting (N. Amer.)	<u>Merluccius</u> <u>bilinearis</u>	Licciardello <u>et al</u> 1982a Peters 1963
Whiting (Aust.)	<u>Sillago</u> <u>ciliata</u>	Sumner & Gorczyca 1981
Witch flounder	<u>Glyptocephalus</u> <u>cynoglossus</u>	Shaw <u>et al</u> 1977
Yellow perch	<u>Perca</u> <u>flavescens</u>	Emerson <u>et al</u> 1966

REFERENCES

- AMPOLA, V.G. and RONSIVALLI, L.J. : (1968) Effect of special handling of haddock on the post irradiation shelf life of haddock fillets. *Fish. Ind. Res.* 4(3), pp. 109-11.
- AMU, L. and DISNEY, J.G. : (1973) Quality changes in West African marine fish during iced storage. *Trop. Sci.* 15 pp. 125-138.
- ANDERSSON, K. and DANIELSON, C.E. : (1961) Storage changes in frozen fish : A comparison of objective and subjective tests. *Fd. Technol.* 15 pp. 55-57.
- AVDALOV, N. and RIPOLL, A. : (1981) Handling, quality and yield of fresh hake. *Bull. Int. Inst. Refrig., Refrigeration Science & Technology*, 1981-4, pp. 71-78.
- BALAKRISHNAN-NAIR, R., THORAMI, P.K. and LAHIRY, N.L. : (1971) Studies on chilled storage of freshwater fish. 1. Changes occurring during iced storage. *J. Food Sci. Technol.*, 8 pp. 53-56.
- BANKS, A. : (1952) Development of rancidity in cold-stored herring; influence of some antioxidants. *J. Sci. Food Agric.*, 3, pp. 250-256.

- BANIK, A.K., CHAUDHURI, D.R. and BOSE, A.N. : (1976) The effect of gamma irradiation on microbial load and sensory evaluation of white pomfret and Indian mackerel fishes. *J. Food Sci. Technol.*, 13(2) pp. 67-69.
- BARASSI, C.A., BOERI, R.L., CRUPKIN, M., DAVIDOVICH, L.A., GIANNI, D.H., SOULE, C.L., TRUCCO, R.E. and LUPIN, H.M. : (1981) The storage life of iced Southern blue whiting (Micromesistius australis). *J. Food Technol.*, 16 pp. 185-197.
- BARNETT, H.J., NELSON, R.W., HUNTER, P.J., BAUER, S. and GRONINGER, H. : (1971) Studies on the use of carbon dioxide dissolved in refrigerated brine for the preservation of whole fish. *Fishery Bull.*, 69 pp. 433-442.
- BARNETT, H.J., NELSON, R.W., HUNTER, P.J. and GRONINGER, H. : (1978) Use of carbon dioxide dissolved in refrigerated brine for the preservation of pink shrimp (Pandalus spp.). *Mar. Fish. Rev.*, 40(9) pp. 24-28.
- BARNETT, H.J., STONE, F.E., ROBERTS, G.C., HUNTER, P.J., NELSON, R.W. and KWOK, J. : (1982) A study in the use of a high concentration of carbon dioxide in a modified atmosphere to preserve salmon. *Mar. Fish. Rev.*, 44(3) pp. 7-11.
- BEUCHAT, L.R., HEATON, E.K. and BOGGESS, J.R. : (1973) Preservation of channel catfish with some selected chemicals. *J. Food Sci.*, 38 pp. 531-535.
- BOSUND, I. and GARNOT, B. : (1970) Effect of pre-cooking on lipid oxidation and storage life of frozen fish. *Lebensmittel-Wissenschaft und Technologie*, 3(4) pp. 71-73.
- BOTTA, J.R. : (1979) Preservation of raw whole short-finned squid (Illex illecebrosus) during the period from catching to processing: Skin color of raw squid and sensory quality of the subsequently cooked squid. *Tech. Rep. Fish. Mar. Serv. Can.* 855.
- BOTTA, J.R., LAUDER, J.T., DOWNEY, A.P. and SAINT, W. : (1983) Chemical and sensory assessment of nonspawning capelin (Mallotus villosus) subjected to long term frozen storage. *J. Food Sci.*, 48(5) pp. 1512-1515.
- BOTTA, J.R., NOONAN, P.B. and LAUDER, J.T. : (1978) Chemical and sensory analysis of ungutted offshore (nonspawning) capelin (Mallotus villosus) stored in ice. *J. Fish. Res. Bd Can.*, 35 pp. 976-980.
- BOTTA, J.R. and SHAW, D.H. : (1975) Chemical and sensory analysis of roughead grenadier (Macrourus berglax) stored in ice. *J. Food Sci.*, 40 pp. 1249-1252.
- BOTTA, J.R. and SHAW, D.H. : (1976) Chemical and sensory analysis of roundnose grenadier (Coryphaenoides rupestris) stored in ice. *J. Food Sci.* 41 pp. 1285-1288.
- CHEUK, W.L., FINNE, G and NICKELSON, R. : (1979) Stability of adenosine deaminase and adenosine monophosphate deaminase during iced storage of pink and brown shrimp from the Gulf of Mexico. *J. Food Sci.*, 44 pp. 1625-1628.
- CRAWFORD, D.L., LAW, D.K. and MCGILL, L.S. : (1972) Shelf life stability and acceptance of frozen Pacific hake (Merluccius productus) fillet portions. *J. Food Sci.*, 37 pp. 801-802.
- CURRAN, C.A., CRAMMOND, V. de B., and NICOLAIDES, L. : (1981b) Spoilage of fish from Hong Kong at different storage temperatures. 2. Quality changes in threadfin bream (Nemipterus japonicus) stored at 0° (in ice), 5 and 10°C. *Trop. Sci.*, 23 pp. 129-145.
- CURRAN, C.A., NICOLAIDES, L., AL-ALAWI, Z. and CRAMMOND, V. : (1981a) Quality changes during iced storage of commercially important fish species from Bahrein. *Trop. Sci.* 23 pp. 253-268.
- CURRAN, C.A., NICOLAIDES, L., POULTER, R.G. and PONS, J. : (1980) Spoilage of fish from Hong Kong at different temperatures. 1. Quality changes in gold-lined sea bream during storage at 0 and 10°C. *Trop. Sci.* 22 pp. 367-382.

- DYER, W.J., FRASER, D.I. and LOHNES, D.P. : (1966) Nucleotide degradation and quality in ordinary and red muscle of iced and frozen swordfish (*Xiphias gladius*). J. Fish. Res. Bd Can., 23 pp. 1821-1833.
- DYER, W.J. and MORTON, M.L. : (1956) Storage of frozen plaice fillets. J. Fish. Res. Bd Can., 13 pp. 129-136.
- DYER, W.J., MORTON, M.L., FRASER, D.I. and BLIGH, E.G. : (1956) Storage of frozen rosefish fillets. J. Fish. Res. Bd Can., 13 pp. 569-579.
- EMERSON, J.A., KAZANAS, N., GREIG, R.A., SEAGRAN, H.L. and KEMPE, L.L. : (1966) Irradiation preservation of freshwater fish. Part I Extension of refrigerated storage life of fresh yellow perch fillets. Fd Technol., 20 pp. 206-208.
- FRASER, D.I., PITTS, D.P. and DYER, W.J. : (1968) Nucleotide degradation and organoleptic quality in fresh and thawed mackerel held at and above ice temperature. J. Fish. Res. Bd Can., 25 pp. 239-253.
- GREIG, R.A. : (1965) Technological investigations of pond-reared fish. 2. Extension of shelf life of buffalofish products through use of antioxidants. Fish. Ind. Res. 2(4) pp. 1-8.
- GREIG, R.A. : (1967a) Extending the shelf life of frozen chub (*Leucichthys hoyi*) fillets through the use of ascorbic acid dips. Fish. Ind. Res. 4(1) pp. 23-27.
- GREIG, R.A. : (1967b) Extending the shelf life of frozen white bass (*Roccus chrysops*) through the use of ascorbic acid dips. Fish. Ind. Res., 4(1) pp. 45-48.
- GREIG, R.A., EMERSON, J.A. and FLICKMAN, G.W. : (1967) Extending the shelf life of frozen cisco (*Coregonus artedii*) products through the use of water soluble anti-oxidants. Fish. Ind. Res., 3(4) pp. 1-10.
- GUTSCHMIDT, J. and PARTMANN, W. : (1977) Sensory and chemical changes of saithe (*Gadus virens* L.) during frozen storage. Archiv. fur Lebensmittelhygiene, 2 pp. 50-56.
- HANSEN, P. : (1960) Danish studies of the storage of wet fish at temperatures close to 0°C. In: Chilling of Fish. Fish Processing Technologists Meeting. Ministry of Agriculture, Fisheries and Food, The Hague, Netherlands, 1960. pp. 151-161.
- HANSEN, P. : (1963) Fat oxidation and storage life of iced trout. I. Influence of gutting. J. Sci. Fd Agric., 14 pp. 781-786.
- HANSEN, P. : (1972) Storage life of prepacked wet fish at 0°C. II Trout and herring. J. Food Technol., 7 pp. 21-26.
- HEATON, E.K., PAGE, J., ANDREWS, J.W. and BOGESS, T.S. : (1972) Changes in the quality of channel catfish held in ice before and after processing. J. Food Sci., 37 pp. 841-846.
- HUSS, H.H. : (1972) Storage life of prepacked wet fish at 0°C. I Plaice and haddock. J. Food Technol., 7 pp. 13-19.
- HUSS, H.H. and ASENJO, I. : (1977) Some technological characteristics of hake from South American waters. In: Proc. Conf. Handling, Processing and Marketing of Tropical Fish, Tropical Products Institute, London. pp. 89-94.
- HUSS, H.H., DALSGAARD, D., HANSEN, L., LADEFOGED, H., PETERSEN, A. and ZITTAN, L. : (1974) The influence of hygiene in catch handling on the storage life of cod and plaice. J. Food Technol., 9 pp. 213-221.
- JHAVERI, S.N., LEU, S.S. and CONSTANTINIDES, S.M.: (1982) Atlantic mackerel (*Scomber scombrus* L.): Shelf life in ice. J. Food Sci., 47 pp. 1808-1810.
- KÖRNER, W. and SCHREIBER, W. : (1980) Shelf life of some antarctic fish during frozen storage. Food Sci. Technol., 13 pp. 326-329.

- KREMSDORF, D.L., JOSEPHSON, R.V., SPINDLER, A.A. and PHLEGER, C.F. : (1979) Gross composition, sensory evaluation and cold storage stability of underutilised deep sea Pacific rat-tail fish, Coryphaenoides acrolepis. J. Food Sci., 44 pp. 1044-1048.
- LAUDER, J.T., MacCALLUM, W.A. and IDLER, D.R. : (1970) Keeping time of frozen redfish (Sebastes marinus mentella) fillets in relation to handling of the raw material and storage temperatures after processing and freezing. J. Fish. Res. Bd Can., 27 pp. 1589-1605.
- LICCIARDELLO, J.J., RAVESI, E.M. and ALLSUP, M.G. : (1980) Extending the shelf life of frozen Argentine hake. J. Food Sci., 45 pp. 1312-1317.
- LICCIARDELLO, J.J., RAVESI, E.M. and ALLSUP, M.G. : (1982a) Stabilisation of the flavour of frozen minced whiting. 1. Effect of various antioxidants. Mar. Fish. Rev. 44 pp. 15-21.
- LICCIARDELLO, J.J., RAVESI, E.M., LUNDSTRON, R.C., WILHELM, K.A., CORREIA, F.F. and ALLSUP, M.G. : (1982b) Time temperature tolerance and physical-chemical quality tests for frozen Red hake. J. Food Qual., 5 pp. 215-234.
- LILJEMARK, A. : (1964) Cold storage of retail packed fillets of mackerel and herring. Food Technol., 18 pp. 380-382.
- LISTON, J., CHAPEL, J.G. and STERN, J.A. : (1961) The spoilage of Pacific Coast rockfish. 1. Spoilage in ice storage. Food Technol., 15 pp. 19-22.
- LUPIN, H.M., GIANNI, D.H., SOULE, C.L., DAVIDOVICH, L.A. and BOERI, R.L. : (1980) Storage life of chilled Patagonian hake. J. Food Technol., 15 pp. 285-300.
- MacCALLUM, W.A., LAISHLEY, E.J., DYER, W.J. and IDLER, D.R. : (1966) Taste panel assessment of cod fillets after single and double freezing. J. Fish. Res. Bd Can., 23 pp. 1063-1081.
- MIYAUCHI D., EKLUND, M., SPINELLI, J. and STOLL, N. : (1964) Irradiation preservation of Pacific Coast shellfish. 1. Storage life of king crab meat at 33° and 42°F. Food Technol., 18 pp. 928-932.
- MIYAUCHI D., PATASHNIK, M. and KUDO, G. : (1975) Frozen storage keeping quality of minced black rockfish (Sebastes spp.) improved by cold water washing and use of fish binder. J. Food Sci., 40 pp. 592-594.
- OSTAVAR, K., SLUSAR, M. and VAISEY, M. : (1967) Preservation of fresh whitefish with gamma radiation. J. Fish. Res. Bd Can., 24(1) pp. 9-19.
- PARTMANN, W. : (1981) Investigations on the storage of packaged rainbow trout (Salmo gairdneri, R.) in air and in carbon dioxide. Fleischwirtschaft, 61 pp. 625-629.
- PATASHNIK, M. : (1966) New approaches to quality changes in fresh chilled halibut. Commer. Fish. Rev., 28(1) pp. 1-7.
- PETERS, J.A., COHEN, E.H. and KING, F.J. : (1963) Effect of chilled storage on the frozen storage of whiting. Food Technol., 17 pp. 787-788.
- PETERS, J.A., MacCALLUM, W.A., DYER, W.J., IDLER, D.R., SLAVIN, J.W., LANE, J.P., FRASER, D.I. and LAISHLEY, E.J. : (1968) Effect of state of rigor and of freezing - thawing processes on storage quality of refrozen cod. J. Fish. Res. Bd Can., 25 pp. 299-320.
- POULTER, R.G. : (1978) Quality changes in fish from the South China Sea: frozen storage of chub mackerel. Proc. Indo-Pacific Fisheries Commission, 18th Session, Manila, Philippines, Section 3, pp. 330-339.
- POULTER, R.G., NICOLAIDES, L. and HECTOR, D.A. : (1978) Quality changes in fish from the South China Sea: Iced storage of chub mackerel, grouper and fusilier. Proc. Indo-Pacific Fisheries Commission, 18th Session, Manila, Philippines, Section 3, pp. 169-185.

POULTER, P.G., SAMARADIVAKRA, B., JAYAWEERA, S.R. and CHIVWASAGAM, H.N. : (1981) Quality changes in three Sri Lankan fish species stored in ice. *Trop. Sci.* 23(2) pp. 155-168.

REPPOND, K.D., BULLARD, F.A. and COLLINS, J. : (1979) Walleye pollock (Theragra chalogramma) - physical, chemical and sensory changes when held in ice and in carbon dioxide modified sea water. *Fishery Bulletin*, 77(2) pp. 481-488.

RIVERO, J.E. : (1981) Quality of frozen hake. *Advances in refrigerated treatment of fish. Bull. Int. Inst. Refrig., Refrigeration Science and Technology*, 1981-4, pp. 301-312.

RYDER, J.M., BUISSON, D.H., SCOTT, D.N. and FLETCHER, G.C. : (1984) Storage of New Zealand jack mackerel (Trachurus novaezelandiae) in ice: chemical, microbiological and sensory assessment. *J. Food Sci.*, 49(6) pp. 1453-1456, 1477.

SHAW, D.H. and BOTTA, J.R. : (1975a) Preservation studies of inshore male capelin (Mallotus villosus) stored in ice. *J. Fish. Res. Bd Can.*, 32 pp. 2039-2046.

SHAW, D.H. and BOTTA, J.R. : (1975b) Preservation studies of inshore male capelin (Mallotus villosus) stored in refrigerated seawater. *J. Fish. Res. Bd Can.*, 32 pp. 2047-2053.

SHAW, D.H. and BOTTA, J.R. : (1977) Chemical and sensory changes in round inshore male capelin (Mallotus villosus) during prolonged storage for 24 months at -23°C. *J. Fish. Res. Bd Can.*, 34 pp. 209-214.

SHAW, D.H., GARE, R.L. and KENNEDY, M.A. : (1977) Chemical and sensory changes during storage of witch flounder (Glyptocephalus cy oglossus) in ice. *J. Food Sci.* 42 pp. 159-162.

SIMMONDS, C.K., LAMPRECHT, E. and HAAS, M. : (1983) Preservation of chilled hake. *Fishing Industry Research Institute, S. Africa. 37th Annual Report.* pp. 29-31.

STANSBY, M.E. and DASSOW, J. : (1949) Storage life of whole and split rockfish fillets. *Comm. Fish Rev.*, 11(7) pp. 1-7.

SUMNER, J.L. and GORCZYCA, E. : (1981) Effect of vacuum packing on the shelf life of fish held either in ice or at 4-6°C. *Advances in the refrigerated treatment of fish. Bull. Int. Inst. Refrig., Refrigeration Science & Technology*, 1981-4, pp. 365-370.

TEENY, F.M. MIYAUCHI, D. and PELROY, G. : (1969) Irradiation of Pacific coast fish and shellfish. 7. Storage life at 33°F of irradiated and prepacked meat of Dungeness crab. *Fish. Ind. Res.*, 5(1) pp. 17-24.

VARGA, S., HIRTLE, W.A. and ANDERSON, W.E. : (1974) A comparison of the storage life and bacterial counts of chilled cod fillets packaged in wooden boxes and heat-sealed plastic containers. *J. Fish. Res. Bd Can.* 31(2) pp. 234-237.

WATERS, M.E. : (1964) Comparison of chemical and sensory tests for assessing storage life of iced calico scallops (Pecten gibbus). *Fish Ind. Res.* 2(3) pp. 5-10.

A LEAP FORWARD IN FRESH FISH MARKETING : THE DARFRESH PACKAGING CONCEPT

John DAVIS, W.R. GRACE LTD, London NW 10 7OH, United Kingdom
Bruno LEPITRE, GRACE Sarl, ESPERNON 28230, France

1. PRESENTATION OF THE DARFRESH VACUUM SKIN PACKAGING (VSP) TECHNOLOGY

The Darfresh VSP system of packaging is the culmination of ten years of research and development of W.R. Grace's plastics for foodstuffs division, the Cryovac Division. Darfresh is the commercial name of the system and is a registered trade mark of W.R. GRACE & Co. It has been developed in the United States on the schedule of charges issued by the European subsidiaries of the Company.

The schedule of charges took into account the diversity and the specificity of the finished products which are sold on the European retail market and took as its objective the packaging in the most attractive form possible of products which are delicate and soft, and which drip, whilst increasing their shelflife significantly.

Conventional packaging methods (vacuum pack and modified atmosphere package) in fact constitute an imperfect response to these two necessities :

- Presentation which makes the product easy and cheap to handle, attractive, and which favours its commercial success,
- Preservation which allows an increase in profitability of distribution networks of perishable products and an improvement in the degree of freshness of the products on display.

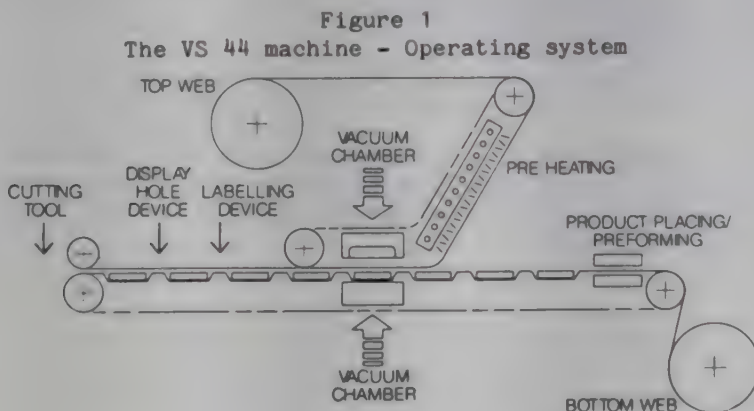
The Darfresh technology is based on two main features :

- the upper and lower packaging films, (top and bottom webs)
- the Darfresh VS 44 machine (fig. 1).

The lower film is a thermoformable compound of 250 to 350 microns which may be printed upon, it can also be metallized, and, apart from its properties which make it a barrier to oxygen, it gives the product its visual rigid support.

As far as fish is concerned, the aluminiumised support was retained to give the product that hygienic image which it all too often lacks in the eyes of the consumer, and also to differentiate it from the white tray overwrapped with PVC stretch film.

The upper film is a 7 layer coextruded and reticulated material, 100 to 150 micron thick. Each layer contributes a certain property to the whole such as being an effective barrier to oxygen (less than 4 cc/m²/24 h to atmospheric pressure), formability and resistance to abrasion or to rupture. The two films have been certified as being suitable for use with foodstuffs.

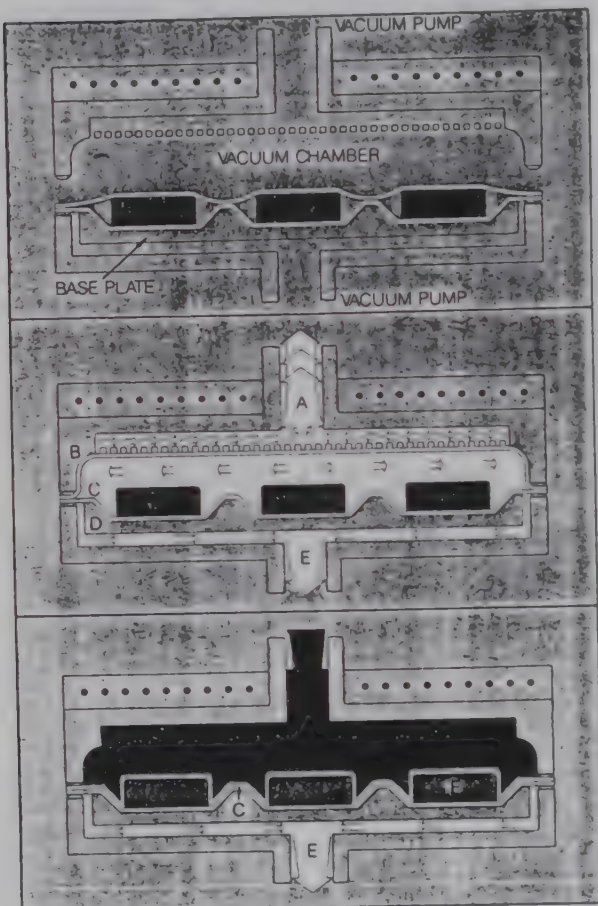


I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985/4

The VS 44 packaging machine allows

- The thermoforming of the lower film to the desired shape at a maximum depth of 27 mm by means of the play of easily interchangeable tooling which adapt to all types of products, as the advance stroke is variable in gradations of 10 mm, between 100 mm and 400 mm, giving genuine packaging versatility
- to form the upper film closely but delicately around every natural contour of the product which serves as a die for the film. This happens in the situation of an industrial vacuum, at 4 residual m.bars and by means of a process of differential vacuuming, with minimal mechanical pressure (fig. 2). The upper film adheres by thermosealing to the surface of the lower film which is not occupied by the product and this film surrounds the product as closely as possible, eliminating all available space for the accumulation of drips.

Figure 2
The Darfresh VSP process



Air is drawn from the top of chamber (A) sucking top film (B) up to roof of heated dome.

The bottom film (C) which rests on a base plate (D) allows air to be evacuated from the rest of the dome through valve (E).

Air is reintroduced through top of the chamber (A) whilst vacuumising continues through lower valve (E).

Difference in air pressure draws heated top film down to form precisely over product (F) and subsequently weld to exposed areas of bottom film (C).

The material cost, from \$ 0.15 to \$ 0.17 per consumer unit according to the thickness of the film used, is more than or the same as that of the conventional technologies but the quality/price ratio of Darfresh packaging is excellent, taking into account its performance.

2. DARFRESH PACK PERFORMANCES

Mr. BOURGEOIS / 1 / found that the calculated shelflife of Darfresh VSP fish was extended by two fold for white fish stored at 0°C along an appropriate cold chain.

Commercial shelf life of Darfresh VSP fish sold on the French market in specific chilling cabinets has been approved by the French health and care authorities (D.S.V.) to be six days after the packaging date. This date marking allows for 24 hours for consumption in the home with storage in a domestic refrigerator.

Advantages in term of presentation of VSP versus all other systems and especially MAP :

- no visible driploss during the commercial shelflife illustrated by Table I below
- less bulky than MAP
- easy control of pack integrity
- better cooling time.

TABLE I

Driploss measured at packaging date plus 5 days expressed as a % of initial weight of lingfish packed in four different systems and stored at 0 to 1°C

MAP (CO ₂ /O ₂ /N ₂ : 40/30/30)	NON SHRINKABLE VACUUM PACK	SHRINKABLE VACUUM PACK	DARFRESH
3,9	3,5	1,7	0,6

Sources : Torry Research Station, Aberdeen, U.C. CANN et al /2/
ADRIA Normandy, M.J. SORNAY.

On top of it, it is worth noting that CASOLARI / ROSSI / 3 / have studied the botulism hazard carried by vacuum packaged fresh fish and its relation to :

- packaging material permeability
- temporary breakages of the cold storage chain.

Conclusions were :

- In all the tests carried out, whatever the clostridium botulinum contamination levels (10⁻¹ / g to 10⁵ / g) fish spoilage took place by far before toxin development
- Uninoculated samples never became toxic
- faster bacterial growth was recorded on samples packaged in oxygen permeable materials than in oxygen barrier materials

But pack performance is not all. The way a new technology is applied to a critical product and takes care of its specificities together with its market profile is also of utmost importance.

3. DARFRESH FRESH FISH CONCEPT : A COMPREHENSIVE APPROACH IMPLYING TOTAL INVOLVEMENT

Darfresh is a new consumer unit packaging system which brings new possibilities and a new future to fresh fish marketing. Through optimal pack presentation and technical performance it brings substantial advantages, to the producer, the retailer and the consumer.

For the Packer :

- Increased turnover by adding value to his product and developing a new product range with the immediate potential of the entire self service retail market.
- Possibility to introduce a consumer branding and quality policy for fresh fish.

For the retailer :

- Opportunity to sell self service fresh fish in all types of outlet without specialized personnel and with no investment other than a special chilling cabinet maintaining a constant 0 / + 2 C temperature.
- Attractive display which has the potential to increase turnover and frequency rates of retail stores.

For the consumer :

- A permanent self service display of ready to cook fresh fish
- a convenient product which guarantees freshness and hygiene
- a product supported by a brand which reassures, guides and informs the consumer.

Total involvement is a necessity when a new technology is being applied to a sensitive application and such a fragile product as fish.

The GRACE CRYOVAC's approach thus included a comprehensive study of the critical factors for an optimum presentation and conservation of fresh fish under Darfresh.

This study has been advised by the best known institutes in Europe on this matter :

- Torry Research Station, Aberdeen, U.K. / 2 /
- Hermetikk Industriens Laboratorium, Stavanger, Norway / 4 /
- Ifremer, Nantes, France / 5 /
- Stazione Sperimentale per la Conserva Alimentari, Parma, Italy / 3 /
- Adria, Quimper, France / 1 /

The result is the precise definition of :

- A code of practice at the production and at the distribution levels (Table II)
- the definition of a specific vertical chilling cabinet maintaining a constant temperature of 0 / + 2° C
- permanent relationship with scientific institutes, organized retail, health & care authorities and consumer associations to secure a professional approach.
- permanent study of the market response to the concept.

Table II

DARFRESH FRESH FISH CODE OF PRACTICE

Selecting	- No more than a total of six days on ice after catch - Discard injured or damaged fish
Processing	- Beheading, eviscerating, smooth brushing of cavities, filleting or slicing under continuous stream of chilled water. Temperature of workshop 8° to 10° C. Maximum processing time : 30 minutes. - Highly hygienic conditions (personnel and premises)
Dipping Draining and drying	- Dipping in a 10 % salt brine at -1° C / 0° C during 1 to 5 minutes depending on species - Draining and drying in a ventilated chilling room or tunnel at 0/+ 1°C during 40 to 60 minutes. These operations aim at reducing the water content of the fish cells by 2 to 3 % and stabilizing the product at an inside temperature of 0 + 2° C before packaging, and thus reducing its tendency to driploss.
Packaging	- Maximum 15 minutes to perform packaging operations until the packed product is returned to a 0 / + 2° C environment - Temperature of the packaging room 6 to 8° C - Highly hygienic environment (personnel and premises).
Shipping	- In appropriate boxes and/or containers to provide a 0/+ 2° C temperature all along the line. - Shipping must occur 36 hours maximum after time of fish landing.
Displaying	- Only in chilling cabinets capable of maintaining a temperature of 0/+ 2° C on every shelf (with a tolerance of + 3,5° C when defrosting).

The Darfresh fresh fish concept has been awarded in September 1985 the approval of the Commission "Quality and Innovation" of the French Ministry of Consumption on the basis of technical and economical results obtained on the French Market in 100 stores supplied by two pilot production sites.

REFERENCES

1. C.M. BOURGEOIS, B. ABGRALL, M. DERENNES : Etude des possibilités de conservation sous vide de poisson frais, ADRIA Quimper 1983.
2. D.C. CANN, G.L. SMITH, N.C. HOUSTON (1983) : Further studies on marine fish stored under modified atmospheres
3. M. ROSSI / M. CASOLARI : Clostridium Botulinum Growth and Toxin development in vacuum packed fresh fish, Stazione Sperimentale per la Conserva Alimentari, Parma 1985.
4. J.M. LUNDE / A.M. JORUM : vacuum packing of fresh fish in different packaging materials, Hermetikk Industriens Laboratorium, Stavanger 1983.
5. L. HAN CHING / A. HADJADJ : Signification et validité des bases azotées volatiles comme critères de détermination de la qualité du poisson frais emballé, IFREMER, Nantes 1983.

POISSON FRAIS : UN SAUT DANS L'INNOVATION - LE CONCEPT D'EMBALLAGE DARFRESH DEVELOPPE PAR W.R. GRACE & Co, EUROPE.

RESUME : Ce concept repose sur une technologie d'emballage dite "seconde peau" particulièrement adaptée aux produits mous et exsudatifs. La machine, VS 44, permet de thermoformer un film barrière rigide sur lequel sont positionnées les portions de poisson prêtes à cuisiner. Elle applique ensuite en ambiance sous vide un film rétractable barrière à l'oxygène 7 couches qui vient épouser les formes du produit et se thermosceller sur toute la surface libre du film inférieur, empêchant toute formation d'exsudat. Le respect d'un cahier des charges strict et notamment d'une chaîne de froid 0 / + 2° C dans les rayons libre service de la grande distribution permet de donner à la gamme de poisson frais une date limite de consommation au 7ème jour qui suit la date de conditionnement.

Le concept Darfresh mis au point avec l'aide des plus grands instituts de recherche Européens a reçu l'agrément de la Commission Qualité et Innovation du Ministère Français de la Consommation.

TABLES RONDES
ROUND TABLE DISCUSSIONS

SUMMARY REPORT ON ROUND TABLE DISCUSSION OF CHILLED STORAGE
SESSION CHAIRMAN

K.J. WHITTLE

Torry Research Station - Aberdeen (United Kingdom)

CONTRIBUTORS

BENNET (UK), BERG (Norway), BLOCKHUS (Norway), GAC (IIR), GIBSON (UK), GOPAKUMAR (India), HERBORG (Denmark), HOWGATE (UK), JUL (Denmark), KARNICKI (FAO), LEARSON (USA), MERRITT (Canada), MUNN (UK), POULTER (UK), SLAVIN (USA) VICKERS (UK)

CHAIRMAN'S INTRODUCTION

The Chairman briefly recalled the papers relevant to the discussion on chilled storage and proposed that the theme for discussion was to consider further the question, "What is shelf-life?", in relation to chilled products. This question had remained unanswered after the papers and discussion of the First Session of the Meeting. Nevertheless, much progress had been made on the factors affecting shelf-life and on methodology in the sensory, chemical and physical fields, illustrated (by way of example) in the use of time/temperature indicators. It was clear also that there was a substantial measure of agreement among researchers on assessment of freshness of iced fish by trained sensory panellists and it ought to be possible to build on this. However, in contrast, no consensus had emerged to select the most appropriate methodologies from those discussed (and others available), to measure the shelf-life of products in a way which is valid not only scientifically and technically, but also realistic both in terms of commercial relevance and of the consumers' perception of product quality. Finally, he reminded participants that Learson and Licciardello had placed 3 recommendations, relevant to both chilled and frozen products, before the Conference in conclusion to their paper, "Literature reporting of shelf-life data. What does it all mean?". Briefly, they had proposed a manual describing standard sensory, and chemical testing methods, their relevance and correlation and, worldwide collaborative testing of procedures.

SUMMARY OF DISCUSSION

During the discussion a number of strongly held opinions emerged which are collated and summarized below.

The simplest definition of the end of shelf-life is the point at which the produce is considered to have become inedible, ie, it is spoilt! At the other end of the scale, the "High Quality Life" can be regarded as the point to which the product retains all its characteristic properties. The equivalent definition in the EC Labelling Directive is "retains its specific properties" and in CODEX and US Grade Standards the cooked product is required to have, "characteristic flavours and no off flavours". Bearing in mind the factory quality controller and others must ensure, in the first instance at least, that spoilage flavours are absent from fish to be processed, it was considered necessary to measure both characteristic and off-flavours. Thus, it was suggested that an agreed methodology for sensory assessment would be the best basis on which to determine shelf-life, with supporting chemical methodology, for instance, of secondary importance. A study had shown that sensory methods are currently the most widely used and accepted because they are both relatively rapid and inexpensive.

From long experience in fisheries inspection and research, it was held to be clearly possible to correlate objective tests with sensory methods but not possible to include consumer preferences in such correlations since the "end of good or acceptable quality" is not a universal applicable criterion. Indeed, some considered that consumers' views could not be assessed, others, that development of objective methods was more important, while others thought that reference to trained panels was more reliable than untrained (consumer) panels. Although a variety of views were expressed, it was generally agreed that the consumer cannot be ignored and that the technologist should not dictate to the consumer what shelf-life should be, particularly since an ideal, non-sensory objective test remained elusive.

I.I.F. - I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

A commercial view recognised that a wide variation in fish quality is available to the processor and that the quality of supplies must be matched to market requirements. For example, some fish may not be of a quality good enough for air, vacuum or modified atmosphere packaging but entirely acceptable for other outlets. Although quality assessment is straightforward most of the time, there is a need for a method for making rapid judgements of the quality of fresh fish. There is also a requirement for a more precise method when the quality is on the borderline of rejection: industry cannot rely on current single indices of freshness since they vary so much! Time-temperature integration may be a solution provided the integral function is appropriate and provided different integrals are available for different products and storage conditions. It was recognised that although microbiological and chemical specifications are widely used, methods are not standardised to give worldwide uniformity. Some took the view that fewer and simpler regulations or standards rather than more and more detailed ones, are preferable.

There was a strong call for a group to set out guidelines on objective and sensory methodology and reporting of data to assess freshness, and to determine what criteria identify the end of shelf-life. Examples were given of current difficulties in comparing results from different sources, to emphasise that the groups should specify the limitations, potential sources of error and the relevant applications of the methods. Guidelines in respect of shelf-life should be as accurate as possible. The question was raised: would any notice be taken of the results of such a study in view of previous experience in the collaborative TMA study? To be useful worldwide, a relatively simple scheme would be needed! Notwithstanding these last two observations, it was suggested that a CODEX, IIR or FAO working party or commission could tackle the problem. However, some felt that any of these would take a long time to come to fruition. The time was ripe for progress on assessment of shelf-life, and a small well-chosen group with clearly defined objectives might be more effective, but would require some source(s) of funding.

SUMMARY REPORT ON ROUND TABLE DISCUSSION ON FROZEN STORAGE
SESSION CHAIRMAN

I.M. MACKIE

Torry Research Station - Aberdeen (United Kingdom)

CONTRIBUTORS

BENNETT (UK), BREMNER (Australia), CORMIER (Canada), GAC (IIR), GOPAKUMAR (India), GRAHAM (UK), HAMILTON (UK), HARDY (UK), HERBORG (Denmark), HOWGATE (UK), JUL (Denmark), KARNICK (FAO), LEARSON (USA), MERRITT (Canada), NEWMAN (UK), OLIVERA (PNG), REID (USA), SCHREIBER (FRG), SCOTT (New Zealand)

CHAIRMAN'S INTRODUCTION

The Chairman's introductory remarks to promote discussion, high-lighted the following points that had emerged during the Meeting, and which raised issues that might prove difficult to resolve.

- 1 Professor Jul's paper which questioned the validity of apparently well established concepts on the grounds that they were not soundly based;
- 2 The importance of taking into account the effects of all processing operations on the storage life of fish and recognising that the quality of even freshly caught fish can vary widely with physiological condition;
- 3 The view that too much attention might be paid to TTT (time temperature tolerance) and not enough to PPP (product, processing and packaging) factors;
- 4 The possibility that modern methods of stock keeping and better temperature control could result in energy savings by allowing storage at somewhat higher temperatures than currently recommended;
- 5 The need for further work on objective indices of change in quality during frozen storage and the fact that, with the possible exception of dimethylamine (DMA) in some gadoids, few met the requirements of industry;
- 6 The observation that hedonic panels were not too discriminating as far as changes in quality during frozen storage were concerned.

SUMMARY OF DISCUSSION

Although no clear final conclusion was reached there was general agreement that world wide standardisation of both objective and sensory testing procedures was desirable.

It was also evident that there were conflicting views on the need to obtain a widely acceptable agreement on the definition of the shelf life of products.

Consumer acceptability, it seemed was a major factor in setting standards and the level of acceptability of a particular product varied not only from country to country but even within a district or region. Therefore, it was not possible to relate consumer acceptability to objective criteria of change. Consideration should be given to recommending procedures for obtaining consumer opinions and a special working group should be set up to resolve this issue. The group would consider the sensory tests that might be used as well as arrange for the work to be done well, hopefully, out of such studies should emerge a measure of the extent of the variation in consumer preference.

The relative merits of objective and sensory tests and the use of time temperature indicators as a means of measuring quality loss on frozen storage were discussed. The majority opinion was that sensory testing must remain as the principal test of storage life but that there was also an urgent need for suitable, complementary objective tests. It was emphasised that sensory data, even from highly trained panels, were still subjective and should be treated accordingly even though a high correlation with non-sensory data was often obtained.

I.I.F.- I.I.R. - Commissions C2, D3 - Aberdeen (United Kingdom) - 1985-4

An industry view was that, for most frozen products, assessment of quality by sensory means was satisfactory but there was a need for more precise objective methods for assessment in borderline cases. It was considered that this requirement was often met by existing chemical, physical or microbiological assessments but that there was a need for a greater harmonisation of methods to provide more precise standards. It was said that present specifications tended to refer mainly to the quality at the time of freezing and, therefore, did not always take account of quality determination during subsequent frozen storage.

There was some discussion on possible funding of a working group after it was made clear that IIR itself did not fund such groups. WEFTA, COST, FAO, EEC and Industry were suggested as possible sponsors. It was assumed that if and when such a group was set up, it would consider harmonisation of both objective and sensory methods of quality assessment.

ROUND TABLE DISCUSSIONS
CONCLUSIONS OF THE ORGANISING COMMITTEE

During both discussions there was a call for the setting up of a group to resolve current problems and difficulties.

Careful, critical scrutiny of the collected papers in the Proceedings of the Specialist Meeting clearly shows the varying degrees to which authors have met the pleas expressed in the Round Table Discussions for compatibility and comparability of experimental approach and presentation of results, and for the identification of short comings in the methodology and its application. It demonstrates the range of interpretation in differing circumstances of such terms as quality, acceptability, preference, keeping time, storage time, storage life, shelf life and potential shelf life. It identifies the scope for improvement and development of research in this area, but it also shows that there is a firm base from which such improvement and development can be encouraged.

Any group set up to examine the problems would need to resolve inter alia the current confusions of terminology and definitions in respect of shelf life, to disentangle the different requirements for standardised methodologies in relation to quality standards; trading specifications and operational quality control, and to relate its conclusions to shelf life and date marking.

The aim of the group would be to set out guidelines on specific aspects, such as:

- 1 objective and sensory methodology,
- 2 the reporting of data to define measures of freshness and quality,
- 3 criteria to identify the end of shelf life for different products.

In these guidelines the group should also specify limitations and potential sources of error, and also indicate relevant applications for the various methods.

To be useful world wide, methods and procedures recommended should be simple although a possible exception may be an accurate method of assessing borderline cases, if any such method could ever be of universal application.

Setting up the group poses both organisational and financial problems. The requirement is for a small, well-chosen group with clearly defined objectives and, preferably, a target timetable. The possibility of an established organisation expert in the field, such as a research laboratory, completing this work for wider approval was not discussed but the Organising Committee feel that this is a possibility that should also be considered.

Funding the group or organisation may remain the outstanding difficulty and since no firm conclusion was reached the suggestion made at the Meeting that the Scientific Council of the IIR should initially deliberate on possibilities remains the most likely first step to resolving this problem.

ISBN 2-903633-31-2

(B 3015)



Printed in Belgium by Ceuterick s.a.
Brusselse straat 153 B-3000 Leuven
A. Struyf Oude Baan 353 B-3000 Leuven

